University of Alberta

Ecophysiological adaptations of black spruce (*Picea mariana*) and tamarack (*Larix laricina*) seedlings to flooding and nutrition stress

by

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ABSTRACT

Black spruce (*Picea mariana* (Mill) B. S. P.), an evergreen conifer and tamarack (*Larix laricina* (Du Roi) K. Koch), a deciduous conifer, are the predominant tree species of boreal peatlands of northern Alberta, Canada. The harsh boreal environment is characterized by low nutrient availability, low soil temperature and high water tables. The rise of the water table creates anaerobic conditions in the root zone where the ammonium form of nitrogen becomes more available while the availability of nitrate decreases. Microsite variation within and between years also results in variable nutrient availability to plants.

In order to understand the mechanisms of flooding tolerance, nutrient uptake pattern, and growth responses, seedlings of both species were subjected to flooding and different forms and levels of nitrogen fertilizers in a series of growth chamber experiments. Results of this project suggest that tamarack is more flood tolerant than black spruce since it maintained a higher gas exchange rate and root hydraulic conductance apparently because of its higher root respiration and greater allocation of carbohydrates to the root. Gas exchange of tamarack was also unaffected by the application of exogenous ethylene though root hydraulic conductivity increased. Additionally, tamarack was able to acclimate morphologically to flooding by producing stem hypertrophy and adventitious roots while black spruce lacked such adaptations.

While nitrate and ammonium uptake was inhibited in flooded seedlings of both species, non-flooded black spruce showed a clear preferential ammonium uptake and tamarack exhibited an equal uptake of both forms of nitrogen. Low supply of nitrogen affected gas exchange of black spruce more negatively than tamarack. On the other hand,

higher nutrient availability for two consecutive seasons did not have a significant positive effect on growth in black spruce while it increased gas exchange rate and biomass production in tamarack dramatically. Maintenance of higher root respiration, root hydraulic conductance, and equal carbon allocation to roots allowed tamarack to withstand flooding while equal uptake of ammonium and nitrate, and higher growth rate in conditions of variable nutrient availability would confer a competitive advantage to tamarack to succeed in the boreal peatland environment.

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List of abbreviations, acronyms and symbols

A_{net}	Net assimilation
ABA	Abscisic acid
ACC	1-aminocyclopropane carboxylic acid
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
CK	Cytokinin
C_2H_4	Ethylene
CO_2	Carbon dioxide
DTPA	Diethylene triamine pentaacetate
DW	Dry weight
EDTA	Ethylenediamine tetraacetic acid
Fe	Iron
FW	Fresh weight
GA	Gibberellic acid
GLM	General Linear Model
g_{s}	Stomatal conductance
IAA	Indole acetic acid
K	Potassium
K_{r}	Root hydraulic conductance
L_{p}	Root hydraulic conductivity
Mn	Manganese
MPa	Megapascal
N	Nitrogen
NAD^+	Nicotinamide adenine dinucleotide
NH_4^+	Ammonium
NO_3^-	Nitrate
O_2	Oxygen
OH^-	Hydroxyl ion
P	Phosphorus
Rubisco	Ribulose bisphosphate carboxylase oxygenase
s.e.	Standard error of the mean

CHAPTER ONE

Introduction

Black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) are predominant tree species in peatlands in North America. They form stands in natural, forested peatlands in Alberta, which have very low productivity (Dang and Lieffers 1989; Lieffers and Macdonald 1990) due to high water table (Lieffers and Rothwell 1986), low nutrient availability (Macdonald and Lieffers 1990; Humphrey 1990), poor soil aeration (Campbell 1980; Mannerkoski 1991) and low soil temperature (Lieffers and Rothwell 1986, 1987). Moreover, they also experience short growing seasons and frequent natural disturbances (e.g., fire) (Rowe and Scotter 1973). It is therefore apparent that the poor climate, edaphic conditions and extreme disturbance regime severely limit the survival and productivity of these tree species in the boreal forests.

In order to cope with limiting growth conditions and to sustain productivity, these boreal trees must have evolved a variety of adaptive strategies. Black spruce and tamarack grow in a variety of infertile peatlands, ranging from ombrotrophic bogs (fed by extreme base-poor precipitation) to minerotrophic- forested fens (fed by more mineral-rich groundwater) (Montague and Givnish 1995). Black spruce, an evergreen conifer, has adapted to the low soil temperature, poor soil aeration and low nutrient supply of peatlands through: a) low nutrient requirements; b) efficient retranslocation of nutrients before needle abscission (Hom and Oechel 1983; Tyrell and Boerner 1987), and c) long-term retention (5-30 years) of photosynthetically active needles (Hom and Oechel 1983;

Tyrell and Boerner 1987), which increases the carbon return per unit of invested nutrient (Small 1972).

Tamarack is a deciduous conifer and sheds its entire foliage each fall (Gower and Richards 1990). The persistence of tamarack in nutrient poor peatlands might be linked to its greater ability for retranslocation of nutrients from senescing needles (Tyrell and Boerner 1987; Matyssek 1986). Although black spruce and tamarack co-occur on moderately minerotropic peatlands, tamarack tends to dominate on wetter, minerotrophic sites while black spruce dominates on weakly minerotrophic to oligotrophic sites (Jeglum and He 1996; Tilton 1977; Kenkel 1987). A correlation between relative dominance and height growth along hydrologic and nutrient gradients suggests that performance differences underlie these species' distributions (Montague and Givnish 1996). Still the dominance of tamarack over black spruce on wetter sites is not well understood. Black spruce and tamarack also possess contrasting growth habits. Black spruce exhibits fixed (preformed) growth. The process of bud set in the previous fall determines its growth. Tamarack has determinate growth of its short shoots but also possesses long shoots displaying indeterminate growth, thus allowing a greater degree of variation of annual growth in response to conditions during the growing season (Clausen and Kozlowski 1967). Evidence also suggests that tamarack has higher photosynthetic nitrogen-use-efficiency and higher total carbon gain than black spruce (Mugasha 1992). Anaerobic conditions limit root growth in both black spruce and tamarack (Lieffers and Rothwell 1987). With decreasing redox potential (usually at 225mV), nitrate availability decreases to zero while ammonium increases (Armstrong et al. 1994). In plants, nitrate assimilation has a greater carbon cost than ammonium uptake (Raven and Smith 1987) but the latter requires root respiration (Chapin et al. 1987). Studies have shown a correlation between gas exchange of tamarack with ammonium availability (Astridge 1996). In addition, the ability of tamarack to transport oxygen to its roots helps to sustain limited respiration under anaerobic conditions (Conlin and Lieffers 1993). This may explain its dominance on wetter sites over black spruce. Thus, it is also expected that tamarack, compared with black spruce, can better take up nitrogen and preferentially use NH_4^+during hypoxic soil conditions.

Low oxygen conditions reduce shoot and root growth by affecting photosynthesis, stomatal conductance and transpiration (Anderson et al. 1984; Pereira and Kozlowski 1977; Pezeshki and Chambers 1986), carbohydrate metabolism and root respiration. Flooding also induces ethylene production (Tang and Kozlowski 1982; Blake and Reid 1981). Morphological adaptations such as stem hypertrophy or formation of adventitious roots are closely associated with increased ethylene production (Blake and Reid 1981). These adaptations also facilitate plant roots to aerate, and eliminate ethylene (Blake and Reid 1981).

Comparative flooding tolerance of black spruce and tamarack has not been investigated. Examining physiological processes and morphological changes, if any, may provide the key to understanding differences in flooding tolerance mechanisms between these two species. Also, examining nutrient uptake and resource allocation under stress can provide useful information on patterns of plant responses and adaptations to changing natural environments.

1.1. Objectives and approach

The objective of this study was to investigate the comparative ecophysiology of black spruce and tamarack. To understand how tamarack exhibits dominance on wetter sites, I studied several physiological processes in tamarack and black spruce including net assimilation, stomatal conductance, water use efficiency, root hydraulic conductance, root respiration and carbohydrate metabolism. I also studied the extent of damage that plants exhibited during flooding in the form of root mass loss, needle necrosis and needle electrolyte leakage (Chapter III and IV). In Chapter V, I used ^{15}N-labelled ammonium (NH_4^+) and nitrate (NO_3^-) to study the nutrient uptake dynamics in both species under flooded and non-flooded conditions. In addition, I also studied the effects of varying nutrient availability (mainly nitrogen) on plant growth (Chapter VI).

The specific objectives of this study were to investigate:

(a) The responses of black spruce and tamarack to flooding and ethylene treatment;

(b) The responses of winter hardened black spruce and tamarack seedlings to flooding;

(c) The preferential uptake of nitrate and ammonium by black spruce and tamarack during flooding; and

(d) Whether indeterminate growth of tamarack could confer greater carbon gain under changing edaphic/nutrient conditions.

Four growth chamber experiments were carried out to address the above objectives (Chapter III-VI). The general discussion and implications of the present study in relation to forest management are presented in Chapter VII.

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CHAPTER TWO

Review of Literature

2.1 Deciduous and Evergreeness

Evergreens with sclerophyllous leaves and thick cuticles are the dominant tree species in the Northern Hemisphere (Larsen 1982). The dominance of evergreen species over trees with a deciduous leaf habit in the harsh environment of the Northern latitudes has been attributed to their greater annual net carbon gain (Mooney 1972; Schulze et al. 1977; Waring and Franklin 1979). The greater leaf life span of evergreen species reduces annual carbon and nutrient requirement to produce new foliage (Chabot and Hicks 1982). Evergreens can also thrive better under conditions of low nutrient availability through their efficient use of nutrients (Aerts 1995; Chapin 1980; Jonasson 1989; Loveless 1961; Monk 1966; Waring and Franklin 1979). Berendse (1994) and Karlson (1992) have shown that the dominance of evergreen species in nutrient-poor environments is due to their low nutrient loss rates. Aerts (1995) demonstrated that plant traits that are associated with low nutrient loss rates lead to low maximum dry-matter production and to low rates of litter decomposition. This suggests a positive feedback between the evergreen habit and low nutrient availability.

Deciduous conifers are also widespread and often dominate parts of the boreal forests (Richards and Bliss 1986). Higher photosynthesis rates (Gowin et al. 1980; Higgins et al. 1987; Matyssek 1986) and greater retranslocation of nutrients from senescing foliage have been attributed for their success in boreal environment along with most of the evergreen conifers.

Black spruce and tamarack are two of the most widely distributed conifer species in North America, particularly in the boreal forests. These two species have contrasting growth habits (Johnston 1990; Viereck and Johnston 1990; Lavoie and Payette 1994). Black spruce is an evergreen conifer with sclerophyllous leaves and generally retains its needles for 5-30 years (Chapin and Van Cleve 1981; Hom and Oechel 1983; Tyrell and Boerner 1987). Tamarack also possesses sclerophyllous leaves but it is deciduous and sheds its entire leaf canopy each fall (Gower and Richards 1990).

Black spruce generally grows on a variety of infertile peatlands and copes well with low nutrient environments (Jeglum and He 1996). Tamarack, however, grows well on wetter, minerotrophic sites (Tilton 1977; Kenkel 1987). Greater retranslocation of nutrients from senescing needles (Tyrell and Boerner 1987; Matyssek 1986) and higher photosynthesis rates (Gowin et al. 1980; Higgins et al. 1987; Matyssek 1986) enable tamarack to survive, grow and regenerate in the adverse boreal environment where evergreens generally dominate. Greater nutrient retranslocation in larch reduces their nutrient demands and favors its deciduous habit (Gower and Richards 1990). Larches and evergreen conifers also have similar total aboveground production in temperate environments (Matyssek 1986). Although Gower and Richards (1990) suggested efficient nutrient retranslocation in larch to be the contributing factor of its widespread occurrence in nutrient- poor peatlands, it does not completely explain the shifts in dominance from deciduous larch to evergreen black spruce or *Thuja or* Cedar along peatland gradients (Givnish 2002).

Montague and Givnish (1996) demonstrated that lower nutrient-acquisition costs will help *Picea* to outgrow *Larix* in infertile soils and *Larix* will outgrow *Picea* on more fertile sites since they have a higher photosynthesis rate, higher leaf N (nitrogen) concentration and a increased advantage of energy allocation to roots. Givnish (2002) has proposed a general model to explain the differential distribution of black spruce vs. larch along the increasing mineral supply gradients in boreal peatlands which involves five major factors, namely: cation availability, N and P (phosphorus) availability, peat decomposition and water-table-depth, length of the growing season and initial stocking density.

According to Givnish (2002) extremely base-poor microsites will favor evergreen species since they have a lower cost of nutrient acquisition and longer growing season. A greater availability of cations (such as K^+) will decrease the energetic advantage of evergreens to uptake such nutrients. Cation supply is expected to increase with soil pH from ombrotrophic bogs to minerotrophic fens and then to rich fens (Wright et al. 1992). Increases in cation supply will also indirectly help to increase the supply of nitrogen and phosphorus in the minerotrophic part of the gradient and favor larch to grow there. High soil N content should accelerate nitrate and/or ammonium production, by lowering peat C: N ratios and stimulating peat decomposition (Malmer 1986). Cations also help buffer the acidification of the peatland soils and groundwater by humic acids and cation exchange by *Sphagnum* (Gorham et al. 1984, 1987). Greater availability/supply of N and P at intermediate pH ranges should favor *Larix* by decreasing its nutrient acquisition costs which is relatively high even with its deciduous habit, by increasing its leaf concentration of N and P and its net assimilation rate. Thus, the shift in dominance from deciduous *Larix* to evergreen *Picea* in the most calcareous peatlands (Wright et al. 1992) can be explained by a decrease in N and P availability in those sites. In addition, the high precipitation/evaporation ratio at boreal and low-arctic latitudes leads to leaching and the formation of very infertile, acid podsols with low rates of nitrogen fixation (Walter 1985; Oechel and Lawrence 1985). Givnish (2002) speculates that leaching and its direct and indirect effects are the key factors that promote the dominance of evergreens at high latitudes.

Shallow water table will reduce nutrient availability by reducing the aerobic root zone and slowing peat decomposition and nutrient release (Ponnamperuma 1984; Lieffers 1988). Minerotrophy fosters shallow water tables in peatlands and is associated with increased oxygenation (Wright et al. 1992). As a result, peat will accumulate slowly and decompose closer to the water table under minerotrophic conditions.

During the early growing season, low temperature and low oxygen soil conditions inhibit water and nutrient uptake in most boreal trees, specifically in black spruce and tamarack considering the environment they are often found in (Kozlowski 1982, 1986; Lieffers and Rothwell 1986; Lieffers and Macdonald 1990; Macdonald and Lieffers 1990). High water table also inhibits the warming of upper peat layers and prevents plants from starting photosynthesis (Lieffers 1988). Thus, a rising water table is expected to favor tamarack by reducing the growing period during spring when evergreen species like black spruce can absorb water and photosynthesize while tamarack awaits bud break.

Givnish (2002) proposes that rooting habit in both deciduous tamarack and evergreen black spruce may play an important role in acclimating to rising water table. Lieffers and Rothwell (1987a,b) and Montague and Givnish (1996) have shown that tamarack roots are deeper than black spruce, which allows the tree to fulfill its greater nutrient requirements by reaching deeper to get access to the mineral rich ground water.

Deep rooting of tamarack compensates for the problem of slow warming of peat layers in the spring, thus favoring its deciduous habit since it awaits bud break, In contrast shallow rooting of black spruce exposes the tree to warm soil earlier and helps its evergreenness since it retains the foliage that can start photosynthesizing immediately. Therefore, it appears that differences in rooting habit are co-adapted to leaf habit. Givnish (2002) suggested that such differences may affect the physiological ability of black spruce and tamarack seedlings to tolerate fluctuating water tables even before local competition for light or rooting space occurs.

2.2 Autecology of black spruce and tamarack

Black spruce is one of the most important pulpwood species of Canada (Viereck and Johnston 1990) and a dominant tree species in the boreal forests of Alberta, Canada (Lieffers and Macdonald 1990). It ranges from northern Massachusetts to northern Labrador on the Atlantic coast, west across Canada to the west coast of Alaska. Its northern limit across Canada and Alaska is about that of the northern tree line (Viereck and Johnston 1990).

Black spruce usually grows on organic soils. The most productive stands are on dark brown to blackish peats, which usually have a considerable amount of decayed woody material (Viereck and Johnston 1990). Black spruce is classified as tolerant to shade but is less tolerant than balsam fir and eastern white-cedar, two common competitors in the eastern part of its range (Viereck and Johnston 1990). The regeneration of black spruce is mostly through seeds; black spruce cones remain partially closed and disperse seed for several years, providing an adequate supply of seeds to reproduce the stand whenever fire occurs. However, vegetative reproduction through layering is also very common, especially when the lower branches of the slow-growing trees are covered by rapidly growing mosses (Stanek 1975). Although some black spruce roots may grow as deep as 60 cm, most roots spread laterally at the moss-humus interface. Most of the root biomass is often found in the upper 20 cm of the organic horizons (Viereck and Johnston 1990). Adventitious roots may develop in areas with rapidly accumulating organic layers.

Black spruce is a post-fire pioneer species on both uplands and peatlands forming even-aged stands (Viereck and Johnston 1990). Black spruce is most prevalent in fire-prone areas such as upland ridges since it can produce seed at an early age (Heinselman 1973). Fire usually results in the immediate reestablishment of black spruce as long as a seed source is available and postfire stands of black spruce are generally even aged. Black spruce becomes dominant on poor peatlands (bog) sites where it encounters little competition from other species. Usually both tamarack and black spruce are the first trees to invade the sedge mat in filled-lake bogs (Viereck and Johnston 1990).

Black spruce is a slow growing species and grows slower than many of the trees with which it is associated. Black spruce thus encounters substantial competition where other co-occurring species are reproduced from sprouts or suckers rather than from seed. Black spruce mostly grows as an understory tree in jack pine and lodgepole pine stands on dry sites, and succeeds the pines in the absence of fire or harvesting (Fowells 1965). Aspen, paper birch, tamarack and black ash often overtop black spruce for many years on well-drained peatland sites before it becomes dominant. In most mixed stands on good peatland sites, black spruce does not compete successfully with balsam fir, eastern white-cedar, red maple, balsam poplar, and black ash after cutting (Fowells 1965). Over much of its natural range in upland or non-bog sites, black spruce is eventually succeeded by balsam fir and eastern white-cedar if undisturbed by fire (Hatcher 1963).

Clearcutting in strips and patches is generally considered to be the best silvicultural system for managing black spruce (Jarvis and Cayford 1967; Johnston 1977; Vincent 1965). An adequate source of reproduction and often some site preparation such as slash disposal is required for satisfactory reestablishment of black spruce after clearcutting. Uneven-aged management can be practiced on poor sites where stands are windfirm and have abundant layering (Johnston and Smith 1983).

Tamarack is also a widespread tree species of the boreal forests in Alberta, Canada (Lieffers and Rothwell 1986; Macdonald and Lieffers 1990). Its wood is heavy and durable, and is used mostly for pulpwood; it is also used for posts, poles, rough lumber, and fuelwood. Tamarack is naturally distributed over a wide range extending from Newfoundland and Labrador west along the northern limit of trees across the Continental Divide to the northern Yukon Territory (Zoltai 1973). It also occurs on bogs underlain by permafrost in Alaska (Zasada 1981). It extends south in the Mackenzie River drainage to northeastern British Columbia and central Alberta; and east to southern Manitoba, southern Minnesota and southern Wisconsin. Given that it is widely distributed, tamarack grows under extremely varied climatic conditions.

Tamarack can grow on a wide range of soil conditions but grows most commonly on wet to moist organic soils (Histosols) such as sphagnum peat and woody peat. Tamarack is more abundant on peatlands than trees characteristic of surrounding uplands since it can withstand high soil moisture, high acidity, and low soil temperature. It grows best, however, on more favorable sites that are moist but well-drained loamy soils along streams, lakes, and swamps; seep areas; and mineral soils with a shallow surface layer of organic matter (Fowells 1965). Tamarack is a characteristic tree species of peatlands, particularly in the southern limit of its range. It occurs on a full range of peatlands from rich swamps (forested rich fen) to raised bogs. It is also quite abundant in poor swamps where the soil water is weakly enriched with mineral nutrients (Heinselman 1970). Tamarack is also fairly common on peatlands in the north (Sims et al. 1979).

Tamarack has good seed years at intervals of 3 to 6 years, with some seed produced in intervening years. Almost 50% of the tamarack seeds that fall may be destroyed by rodents (Fowells 1965). Combined with seed loss to fungal and bacterial infection, the germination rate is only 4-5 % (Fowells 1965). Vegetative reproduction through layering is the dominant reproductive mode for tamarack along the northern limit of trees in Canada and Alaska (Elliott 1979; Zasada 1981).

Tamarack generally has a relatively shallow (still deeper than black spruce) but spreading root system. Roots may spread over an area greater in radius than the tree height on favorable sites but are only up to 61 cm deep (Johnston 1990). Peatland tamaracks have wide root systems but do not form taproots. All larches (including tamarack) are very intolerant of shade (Olaczek 1986). Although tamarack can tolerate some shade during the first several years (Johnston 1983; Zasada 1981), it must become dominant to survive. Tamarack is a pioneer tree species on open unburned bogs and burned organic soil (Eyre 1980). Since tamarack is very shade intolerant, it does not become established under its own shade. As a result, black spruce eventually succeeds tamarack on poor (bog) sites, because it is more shade tolerant than tamarack.

Gower and Richards (1990) found tamarack having similar above- ground production to co-occurring evergreen conifers. Tamarack exhibits greater height growth than black spruce (Mead 1978) even on crowded sites and under very limiting conditions (Montague and Givnish 1996; Bares and Wali 1979). Tamarack has high nutrient-use-efficiency and can retranslocate nutrients efficiently and allocate less carbon for below-ground growth since most larches exhibit lower fine root growth than evergreen conifers because of greater carbohydrate requirements by larches for construction of a full complement of foliage compared to the single age-class of foliage produced by evergreen conifers (Lyr and Hoffman 1967). Thus, its lighter-weight canopy allows for greater allocation to height growth (Matyssek 1986; Tyrell and Boerner 1987; Gower and Richards 1990; Montague and Givnish 1996).

Photosynthetic rates of tamarack (expressed on a dry weight basis) are often twofold higher than current needles of evergreen conifers (Fry and Phillips 1977; Gowin et al. 1980; Higgins et al. 1987; Matyssek 1986). Evidence also suggests that tamarack has higher nitrogen-use-efficiency and higher total carbon gain than black spruce (Mugasha 1992). However, the greater total leaf mass of evergreens such as black spruce compensates for their low rates of photosynthesis per unit leaf mass (Givnish 2002). Tamarack, being a deciduous conifer, has a lower root construction/maintenance cost when soil becomes very dry, wet or cold (Givnish 1979, 1986a, b) since it can reduce transpiration rates significantly in adverse conditions. Furthermore, tamarack requires no leaf respiration during an unfavorable season (Givnish 2002).

Infertile soils generally reduce the maximum rate of photosynthesis by reducing the optimal level of leaf nitrogen (Mooney and Gulmon 1979). Thus, soil infertility also reduces the absolute seasonal differences in net photosynthesis and would favor the dominance of evergreens in boreal forests (Givnish 2002). However, with increasing soil fertility the dominance shifts from evergreens to deciduous plants (Fox 1992; Aerts et al. 1991; Nams et al. 1993; Givnish 2002).

Stem sapwood serves for water storage in conifers to meet transpirational demands up to several days (Schulze et al. 1985; Waring et al. 1979). Larches can store greater volume than co-occuring evergreen conifers (Gower 1987). Thus, Gower and Richards (1990) speculated that larches would be able to sustain high transpiration rates, and consequently high photosynthesis rates when soil water availability is high since they have a greater sapwood volume, sapwood area:leaf area ratio, and lumen diameter than evergreen conifers.

Even-aged management, with some adaptation of clearcutting or seed-tree cutting is generally considered the best silvicultural system since tamarack seeds germinate better in the open and the seedlings require practically full light to survive and grow well (Johnston 1990).

2.3 Flooding responses in conifers

Flooding affects many plant physiological processes through depletion of soil oxygen, modification of soil structure, accumulation of carbon dioxide and by inducing anaerobic decomposition of the rhizosphere. Although there is a rather extensive literature on effects of flooding on growth of woody plants (Gill 1970; Rowe and Beardsell 1973; Kozlowski 1982), only a few quantitative studies have been conducted with gymnosperms. The majority of gymnosperms are not very flood tolerant, although responses vary widely among species (Hunt 1951; Poutsma and Simpfendorfer 1963; Minore 1968; Coutts and Philipson 1978a, 1978b), with age of trees (Ahlgren and Hansen 1957), soil factors (Gill 1970), and timing and duration of flooding (Kramer and Kozlowski 1979).

Flood intolerant species respond during the early phases of flooding by rapidly closing their stomata (Kozlowski and Pallardy 1979; Pereira and Kozlowski 1977; Tang and Kozlowski 1982). Flood tolerant species, on the contrary, are able to acclimate to the flooding conditions and reopen their stomata after a period of flooding which subsequently induces the formation of hypertrophied lenticels and adventitious roots on submerged portions of stems (Sena Gomez and Kozlowski 1980). Stomatal reopening leads to recovery of plants through accelerated photosynthesis (Tang and Kozlowski 1984). Hypertrophied lenticels may contribute to flood tolerance by facilitating exchange of dissolved gases in the floodwater and by serving as openings through which toxic compounds associated with anaerobiosis (such as acetaldehyde, ethanol, and ethylene) are released (Chirkova and Gutman 1972; Kozlowski 1982). Variation in flooding tolerance among and within conifer species has been linked to root and stem aeration capacity, the potential for stress-induced production of aerenchyma in existing or newly formed roots, and the formation of adventitious roots (Philipson and Coutts 1978; Sanderson and Armstrong 1980; Pezeshki 1991).

Black spruce and tamarack grow in a variety of infertile peatlands, ranging from ombrotrophic bogs (fed by extremely base-poor precipitation) to minerotrophic forested fens (fed by mineral-rich groundwater) (Montague and Givnish 1996). Although both tamarack and black spruce (Ahlgren and Hansen 1957) are presumed to be flood tolerant based on their natural occurrence, tamarack often occurs on microsites with higher water table than those occupied by black spruce (Bares and Wali 1979; Karlin and Bliss 1984; Kenkel 1987; Jeglum and He 1996; Montague and Givnish 1996).

2.3.1. Morphological responses of black spruce and tamarack

Black spruce can adjust to rising water tables by producing layering from branches, and less commonly by root sprouting (Fowells 1965). Formation of hypertrophied lenticels on submerged stems and roots of tamarack (Hahn et al. 1920) have been reported during flooding. There is evidence that lenticels at the stem base are important points of oxygen entry (Coutts and Armstrong 1976) which facilitate aeration of submerged roots (Vartapetian and Jackson 1997). Furthermore, potentially toxic compounds associated with anaerobiosis such as acetaldehyde, ethanol, and ethylene are released from plants through these lenticels (Chirkova and Gutman 1972). Evidence suggests that in some species morphological responses to flooding, such as hypertrophy of lenticels and formation of adventitious roots, are associated with increased production of ethylene (Blake and Reid 1981; Newsome et al. 1982; Tang and Kozlowski 1982a, 1982b). Ethylene also induces the development of root initials that were already present (Kawase 1981). Kawase (1978) demonstrated that significantly increased ethylene production in parts of stems that were maintained in an oxygen-free atmosphere. Ethylene concentration was highest in the stem parts exposed to anaerobiosis, and was progressively lower on unflooded parts of the stem.

The close association of adventitious root formation and stomatal reopening in some species suggests that these aerenchymatous roots may play a key role in the recovery of gas exchange from flooding stress (Tang and Kozlowski 1984). This mechanism enables absorption of O_2 by aerial tissues, basipetal movement of oxygen through stems, and diffusion of oxygen out of the roots to oxidize the rhizosphere. Oxygen can directly oxidize the rhizosphere or enzymatic activity of root-associated microbes may contribute for rhizospheric oxidation (Armstrong 1975). Oxidation of the rhizosphere enhances absorption of minerals by roots and transforms soil-borne toxins to less harmful compounds (Hook et al. 1970; Hook and Brown 1973). Thus, morphological adaptations such as formation of adventitious roots have been attributed to the high nutrient-uptake efficiency of flood-tolerant plants (Kozlowski 1997). In addition, flood-induced adventitious roots increase water absorption by roots (Jackson and Drew 1984; Tsukahara and Kozlowski 1985) and increase the supply of root synthesized gibberellins and cytokinins to the leaves (Reid and Bradford 1984) for continual leaf growth and delay of senescence.

2.3.2. Physiological responses

Soil flooding affects many physiological processes including gas exchange, water relations, carbohydrate metabolism, mineral uptake, and hormone synthesis (Drew 1983; Kozlowski 1997; Nilsen and Orcutt 1996; Pezeshki 1994). Flooding of soil is generally followed by a rapid reduction in photosynthesis in many plants, including conifers (Kozlowski 1997; Pezeshki 1994). The rate of photosynthesis has been shown to reduce within hours to a few days after flooding is initiated (Zaerr 1983; Phung and Knipling

1976). The early reduction in the rate of photosynthesis is correlated with stomatal closure, resulting in decreased CO_2 absorption by leaves (Pezeshki et al. 1996a). Stomatal closure in response to flooding has been demonstrated in many species. Stomata reopened and the rate of photosynthesis increased when floodwater was removed (Davies and Flore 1986c). However, the capacity for stomatal reopening varies with species and the duration of the flooding (Kozlowski 1997). Stomatal closure in flooded plants was found to be associated with a decrease in root hydraulic conductivity (Andersen et al. 1984b; Davies and Flore 1986a) resulting in reduced rates of transpiration and photosynthesis (Drew 1983).

Reductions in the activity of the carboxylation enzyme (Rubisco – ribulose bisphosphate carboxylase oxygenase) may also contribute to the reduced photosynthetic capacity during flooding (Dreyer et al. 1991; Gardiner and Hodges 1996). The specific mechanism by which reduction in carboxylation activity is mediated is unknown (Gardiner and Krauss 2001).

There are at least two possible mechanisms by which root hypoxia could disrupt Rubisco activity and thereby reduce photosynthetic capacity. The first pathway involves a chemical signal, ABA (Abscisic acid), which is produced in the hypoxic roots and transported through the xylem to the leaves (Neuman and Smit 1991). ABA has been shown to reduce carboxylation efficiency (Ward and Bunce 1987). The second pathway by which root hypoxia could reduce photosynthetic capacity involves carbohydrate signaling in leaves. It is believed that flooding inhibits assimilate translocation to roots resulting in an accumulation of foliar carbohydrates (Schumacher and Smucker 1985), which thus inhibits expression of the Rubisco gene (Moore et al. 1999).

Other probable factors responsible for restricting photosynthetic activity include deficiency of cytokinin in the leaf (Bradford 1982) and premature leaf senescence. Photosynthetic reduction in response to flooding was attributed to ABA accumulation and cytokinin depletion, which resulted in reduced ribulose bisphosphate regeneration capacity in leaves of tomato plants (Bradford 1982).

Sulfide uptake under flooding has been documented for several species (Carlson and Forrest 1982; Pearson and Havill 1988). The inhibitory effects of sulfide on photosynthesis of Lemnaceae species has been reported by Takemoto and Nobel (1986). The photosynthetic capacity decreases because of alterations in activity of photosynthetic enzymes (Khan and Malhotra 1982; Kropff 1987), disruption of light reactions (Shimazaki and Sugahara 1981), and/or photophosphorylation (Wellburn et al. 1981).

2.3.3. Root water uptake and leaf-water relations

Root water uptake and transport are inhibited in many species during flooding, possibly due to the inhibitory effects of hypoxia on root permeability and hydraulic conductivity (L_p) (Anderson et al. 1984; Smit and Stachowiak 1988; Everard and Drew 1989). Reduction in the diameter of the metaxylem and protoxylem vessels during flooding restricted axial conduction of water movement in both flood-tolerant and –sensitive cultivars of wheat resulting in reduced root hydraulic conductivity (Huang et al. 1994).

Plant root water uptake requires oxygen (Rowe and Beardsell 1973). Kramer (1983) demonstrated that the toxic effect of high CO_2 concentration in flooded soils was the key factor for reducing root hydraulic conductivity. However, Hunt et al. (1981) attributed the reduction in L_p to accumulation of ethylene in the soil and plant. Flood induced reduction in hydraulic conductivity was followed by stomatal closure in *Vaccinium corymbosum* (Davies and Flore 1986a; 1986b) and *Citrus* (Syvertsen et al. 1983). The effects of soil flooding on leaf water status and stomatal conductance vary with species or cultivars differing in flooding tolerance. A significant reduction in leaf water potential (ψ) and stomatal conductance (g_s) has been reported in a flood-sensitive cultivar of wheat but not in a tolerant cultivar (Huang et al. 1994). In *Melaleuca quinquenervia* (Sena Gomez and Kozlowski 1980a) and *Populus deltoides* (Regehr et al. 1975), however, flood induced stomatal closure was not associated with a significant reduction in leaf turgor or leaf water potential. Thus, factors other than water deficit may induce stomatal closure and inhibit stomatal conductance during flooding.

However, Else et al. (2001) reported that stomatal closure and slower leaf expansion in flooded *Ricinus communis* were due to a decrease in L_p that reduced leaf water potential despite diminished delivery of ABA from the roots to shoots in xylem sap. A decline in transport of K^+ (Sojka and Stolzy 1980) and cytokinin (Railton and Reid 1973) to shoots may also contribute to stomatal closure in flooded plants.

2.3.4. Carbohydrate metabolism

Carbohydrates are the primary energy storage compounds in plants and their allocation pattern and translocation rate are often associated with flooding tolerance (Huang and Johnson 1995). Environmental conditions, species and genotype variation within the same species of plants (Setter et al. 1987) dictate the rate and extent of increase in sugar content when subjected to hypoxia. Soluble carbohydrates such as sucrose, glucose or fructose are of particular importance because of their direct relationship with respiration.

Assimilate translocation to roots is reduced in plants grown under low oxygen conditions (Sij and Swanson 1973; Qureshi and Spanner 1973; Schumacher and Smucker 1985). Schumacher and Smucker (1985) demonstrated that the translocation of carbon to anoxic roots was less than 50% of that to aerated roots in *Phaseolus vulgaris*. Furthermore, most recent photosynthesized carbon translocated to anoxic roots was excluded from respiratory metabolism. Thus, the reduction in carbon translocation to hypoxic roots may lead to low carbohydrate availability (Web and Armstrong 1983). Oxygen deficient conditions also inhibit root elongation due to low carbohydrate reserves in the root tips (Webb and Armstrong 1983). Root apices are most likely to suffer more injury than mature roots under low-oxygen stress because of their low sugar content (Thomson et al. 1990).

Carbohydrate concentrations remained the same or increased in roots under hypoxic conditions for several species (Benjamin and Greenway 1979; Limpinuntana and Greenway 1979; Papenhuijzen 1983; Barret-Lennard et al. 1988; Huang and Johnson 1995), however, the changes were species specific and varied among genotypes within species (Setter et al. 1987). Barclay and Crawford (1983) have shown that tolerance of plants to flooding is dependent on the available carbohydrate reserves in the roots, which facilitates ion uptake through a supply of energy (Setter et al. 1987). However, Limpinuntana and Greenway (1979) and Benjamin and Greenway (1979) have suggested that sugar accumulation in roots is a consequence of reduced root growth rather than adaptation to waterlogging. Restricted synthesis of polysaccharides or accelerated rates of glycholysis of cell water constituents may lead to increased sugar accumulation in the roots at low oxygen concentration (Heide 1963). Nutrient deficiency could also be related to increased sugar accumulation (Huang 2000).

The role of carbohydrate status of roots to confer hypoxia tolerance has been demonstrated by supplying exogenous carbohydrate to roots grown under low oxygen conditions. Exogenous supplies of carbohydrates to roots significantly enhanced their survival at low-oxygen stress (Waters et al. 1991; Web and Armstrong 1983). Anoxia tolerance of maize seedlings was enhanced by adding glucose to the rooting medium, which increased the ATP content in the root tips (Waters et al. 1991). Furthermore, supply of exogenous glucose prevented root ultrastructural injury under low oxygen conditions (Webb and Armstrong 1983).

Flooding also reduces aerobic respiration of roots (Nilsen and Orcutt 1996; Drew 1983; Lambers et al. 1978; Huang and Johnson 1995; VanTaoai et al. 1995). However, the magnitude of reduction varies with plant species and cultivars. The ability of plants to minimize the reduction in root respiration is associated with their ability to tolerate the adverse effects of flooding. Roots of some species switch to anaerobic respiration when subjected to root zone anoxia. Anaerobic respiration produces inadequate ATP that can sustain growth and cell maintenance (Nilsen and Orcutt 1996; Drew 1983). In aerobic respiration each glucose molecule produces 36 molecules of ATP while only 2 molecules of ATP is produced in anaerobic respiration (Nilsen and Orcutt 1996). Anaerobic respiration could result in cell injury and death through the accumulation of acetaldehyde and ethanol since glucose cannot be completely oxidized to CO_2 and water.

2.3.5. Nutrition uptake and accumulation during flooding

Flooding reduces the uptake rate of macronutrients such as nitrogen (N), potassium (K), and phosphorus (P) in many woody plants (Rosen and Carlson 1984; Smith and Bourne 1989; Larson et al. 1992) and thereby their supply to shoots (Drew and Sisworo 1979; Orchard et al. 1986; Huang et al. 1995). A reduction of nutrient supply to the shoots results in reduction in chlorophyll content and photosynthetic capacity and initiation of premature senescence (Drew 1983; Huang et al. 1995; Trought and Drew 1980).

The reduction in nutrient uptake induced by flooding may be due to restriction of root growth, the inefficiency of anaerobic respiration in providing adequate energy for active ion uptake, and increased permeability of cell membranes in roots (Drew 1983). These impediments often operate concurrently. Denitrification and leaching often results in reduced N availability and therefore reduced uptake under soil hypoxia. The availability of phosphorus is also reduced in acidic soils.

In contrast, availability and uptake of Fe and Mn is often increased by flooding since ferric and manganic forms are converted to soluble ferrous and manganous forms (e.g., Jones and Etherington 1970). However, abundant ferrous and manganous ions in flooded soils may be toxic to some plants (Crawford 1989).

Translocation of nutrients between plant parts is also affected by flooding. Increased amounts of N and P accumulated in flooded roots of wheat (Huang et al. 1995) and in loblolly pine (McKee et al. 1984) compared to shoots. These reductions in nutrient transport from roots to shoots were attributed to reduced water uptake (Nilsen and Orcutt 1996).

Flooded soil is dominated by ammonium since the availability of nitrate decreases with decreasing redox potential (Armstrong et al. 1994). Nitrogen uptake in plants occurs either in the form of NO_3^- or NH_4^+. Nitrate assimilation has a greater carbon cost than NH_4^+ uptake (Raven and Smith 1976) but the latter requires root respiration (Chapin et al. 1987). In well-aerated nutrient solutions, NO_3^- and NH_4^+ uptake rates of coniferous tree roots differ considerably and depend on both concentrations and proportions of the nitrogen forms supplied (van den Driessche 1971). At similar external concentrations the uptake rates of NH_4^+ are usually considerably higher than those of NO_3^- (Boxman and Roelofs 1988; Scheromm and Plassard 1988). In annual plants (Lee and Drew 1989) and coniferous trees, uptake rates of NO_3^- are severely depressed by high NH_4^+ concentrations (Scheromm and Plassard 1988).

Since ammonium is toxic to a large number of plants (Bennet 1974), rapid accumulation can lead to a serious reduction in growth. However, some conifers have been shown to grow very well with ammonium, sometimes preferring a mixture of ammonium and nitrate (Krajina et al. 1973; van den Driessche 1971). It was reported that when exposed to equal concentrations of both NH_4^+ and NO_3^- loblolly pine absorbed over 80% of its total N as NH_4^+ while ponderosa pine absorbed less than 30% as NH_4^+ (Griffin et al. 1995). The presence of substantial amounts of free ammonium has been noted in *Picea abies* and *Pinus nigra* foliage without apparent negative effects on growth rates (Clement and Gessel 1985), suggesting that some conifers may be tolerant of free ammonium in the symplast.

2.3.6. Hormone synthesis

Ethylene is produced in the roots of flooded plants (Blake and Reid 1981) and translocates to the shoots. ACC (1-aminocyclopropane-1-carboxylic acid; a precursor of ethylene) is synthesized in roots at an accelerated rate during hypoxia (Bradford and Yang 1980). Increased accumulation of ethylene in flooded plants has been associated with reduced shoot elongation, increased diameter growth, leaf wilting, epinasty, chlorosis, and premature senescence (Nilsen and Orcutt 1996). Flooding also increased ABA concentration in the leaves of bean (Wadman-van Schravendijk and van Andel 1986), pea (Jackson et al. 1988) and alfalafa (Castonguay et [illegible] 1993). However, whether ABA originates from the leaves through synthesis or is produced in the roots and transported to the leaves remains uncertain. Jackson et al. (1988) suggested that ABA accumulates in the leaves of flooded plants because of reduced phloem transport. Hoffmann-Benning and Kende (1992) observed that ethylene reduces ABA levels in the intercalary meristems and part of the elongation zone in rice plants suggesting that ethylene and ABA together may interact to influence stomatal opening.

Flooding causes an increase in auxin concentration in the shoots and reduced levels in roots of plants (Nilsen and Orcutt 1996). McNamara and Mitchell (1991) demonstrated, using IAA and ethylene transport inhibitors and antagonists respectively, that the anoxic condition created at the level of the flood line causes an accumulation of IAA as a result of transport inhibition due to high levels of ethylene in the root zone. Hypoxia also caused a reduction in the cytokinin flux (conc. x mass flow rate of sap) from roots to shoots in the xylem (Neuman et al. 1990). The effect of flooding on gibberellin levels was variable. While Neuman et al. (1990) observed a small but significant reduction of GA_1 and GA_3 in poplar under hypoxia, Hoffmann-Benning and Kende (1992) reported that GA_1 and GA_{20} increased fourfold (after 3 h of submergence) and threefold (after 24 h submergence) in intercalary meristems and internode elongation zones of adult deepwater rice plants.

Flooding induced physiological changes/processes in plants are presented in the following schematic diagram:

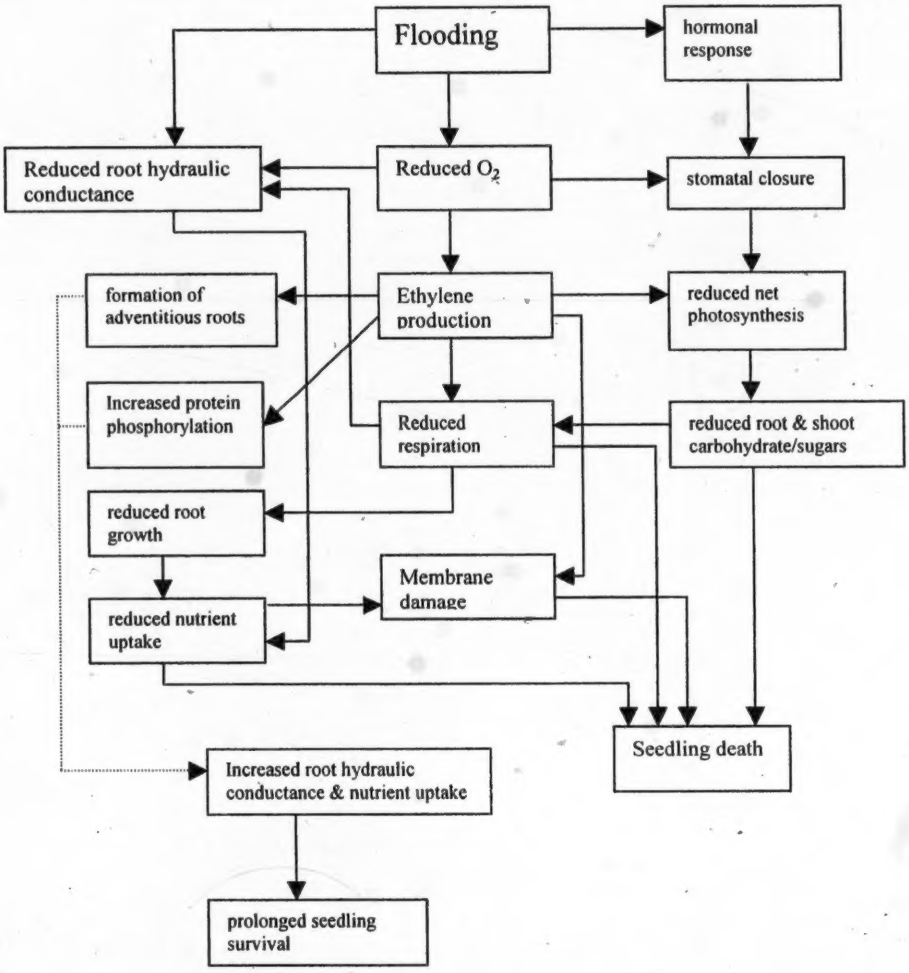
Flooding
hormonal response
Reduced root hydraulic conductance
Reduced O_2
stomatal closure
formation of adventitious roots
Ethylene production
reduced net photosynthesis
Increased protein phosphorylation
Reduced respiration
reduced root & shoot carbohydrate/sugars
reduced root growth
Membrane damage
reduced nutrient uptake
Seedling death
Increased root hydraulic conductance & nutrient uptake
prolonged seedling survival

Figure 2.1. Schematic diagram of flood induced physiological processes in plants.

In summary, flooding affects the hormonal balance in plants. The increase in ABA concentration induces stomatal closure in flooded plants. Stomatal closure leads to reduced photosynthesis rate, which in turn declines the root and shoot carbohydrate content and seedling death. Flooding, on the other hand, reduces oxygen content and leads to ethylene production in plants. Reduced oxygen in the root zone also leads to reduced root respiration and thereby affects plants ability to uptake water. Flooding also reduced root hydraulic conductance, which results in reduced nutrient uptake and enhances seedling mortality. However, ethylene enhances protein phosphorylation and increase in root hydraulic conductance in flooded plants. Ethylene induced production of adventitious roots in flooded plants also help in increased nutrient uptake and could enhance seedling survival during prolonged flooded period.

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CHAPTER THREE[*]

Responses of black spruce (*Picea mariana*) and tamarack (*Larix laricina*) to flooding and ethylene

3.1 Introduction

Black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) are the dominant tree species in the boreal peatlands in Alberta, Canada. Natural stands of these species have very low productivity (Lieffers and Macdonald 1990). These organic sites are characterized by shallow water table and cold substrate temperatures (Van Cleve et al. 1983) and undergo significant variation in depth of water table during the growing season. The growth of roots of these species is generally confined to surface layers above the average water table depth, with deeper roots being pruned off by anaerobic conditions (Boggie and Miller 1976, Mannerkoski 1985, Lieffers and Rothwell 1986). Oxygen supply to tree roots decreases significantly when the water table rises close to the peat surface (Kozlowski 1984, Mannerkoski 1985).

Low oxygen (hypoxia-inducing) conditions, characteristic of flooded soils, result in a rapid reduction in transpiration, stomatal conductance and photosynthesis of many woody plants (Anderson et al. 1984, Levan and Riha 1986, Pereira and Kozlowski 1977, Pezeshki and Chambers 1986) resulting in decreased tree productivity. As an evergreen with sclerophyllous leaves, black spruce is considered a bog-adapted plant. Tamarack also has sclerophyllous leaves but is deciduous (Tyrell and Boerner 1987). Although black spruce and tamarack co-occur on moderately minerotropic peatlands, tamarack tends to dominate on wetter, minerotropic sites (Jeglum and He 1996, Tilton 1977, Kenkel 1987). A correlation between relative dominance and height growth along hydrologic and nutrient gradients suggests that performance differences underlie these species' distributions among microsites within peatlands (Montague and Givnish 1996). Still the dominance of tamarack over black spruce on wetter sites is not well understood. The ability of tamarack to transport oxygen to its roots and sustain limited respiration under anaerobic conditions (Conlin and Lieffers 1993) may play a role.

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Flooding triggers ethylene accumulation in plants (Tang and Kozlowski 1984*a* and *b*, Blake and Reid 1981) and plant physiological processes such as net assimilation, transpiration, and stomatal conductance are inhibited by elevated levels of ethylene (Govindarajan and Poovaiah 1982, Kays and Pallas 1980, Pallas and Kays 1982). Ethylene produced during flooding (Kawase 1976) was the likely factor responsible for promoting the outgrowth of adventitious roots in tamarack (Hahn et al. 1920). Flood tolerance and emergence of adventitious root often occur simultaneously (Clemens et al. 1978, Sena Gomez and Kozlowski 1980a, 1980b).

Although several studies have been conducted to examine flooding tolerance of black spruce, no studies have compared flooding tolerance of black spruce and tamarack. Therefore, we examined the effect of 4 weeks of soil flooding on gas exchange, root hydraulic conductance, ethylene evolution and membrane damage in black spruce and tamarack. We also examined the effect of exogenous ethylene on these physiological processes. We hypothesized that flood tolerance of tamarack is conferred by its greater tolerance to ethylene accumulation.

3.2 Materials and Methods

3.2.1 Plant material and treatment application

Six-month-old black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) seedlings were used in the flooding experiment. Seeds were obtained from the Alberta Tree Improvement and Seed Center (ATISC acc #4042). The seeds were collected from ten different sixty-year-old tamarack trees in the natural forests of Calling Lake, Alberta (latitude 55° 03' and longitude 113° 14'). Seeds were stratified for three weeks at 4^0C and seeds were germinated in Petri-dishes. One week after germination, seedlings were transplanted to Spencer-Lemaire (300ml) containers (Spencer-Lemaire Industries Ltd. Edmonton, AB. Canada) containing (1:1:1; peat:moss:vermiculite) planting medium Pro-Mix BX (Premier Horticulture Inc., Riviere-du-Loup, Que. Canada) and placed in the greenhouse under 21 ± 3°C temperature and 16-h photoperiod. Seedlings were watered to run off every other day and fertilized once a week with 30-10-10 N-P-K fertilizer (Plant-prod®- Plant Products Co. Ltd., ON, Canada). Seedlings were transferred to 5" pots when they were three months old.

One week prior to the start of the experiment, seedlings were transferred to a controlled growth chamber with 21°C/18°C day/night temperature, 65% relative humidity and 16-h photoperiod with photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} provided by fluorescent lamps.

3.2.2 Treatment application and experimental design

Seedlings from both black spruce and tamarack were randomly placed in buckets where flooding was imposed by submerging the seedlings to a root-collar level. Perforated buckets were used for control (non-flooded) seedlings to ensure adequate drainage. Data were collected on day 2, 5, 8, 11, 14, 18, 22 and 28 after treatment imposition. On each measurement day, the various physiological parameters were measured on five randomly selected seedlings from each species x treatment combination. Only one seedling per species was withdrawn from a given bucket on a given measurement day. A total of 160 seedlings (N) were used in this experiment. The experiment was a split-split-plot design (between subjects: flooding, within-subjects: species and time) and buckets were considered nested within treatments.

The statistical model is as following:

$$Y_{ijklm} = \mu + F_i + B_{j(i)} + S_k + FS_{ik} + B_{j(ik)} + T_l + TF_{il} + TS_{kl} + TFS_{ikl} + E_{ijklm}$$

Where, Y_{ijklm} = dependent variable (physiological parameter e.g., net assimilation, stomatal conductance, electrolyte leakage etc.), μ = overall mean, F = flooding treatment ($i = 1,2$), B = bucket within flooding treatment ($j = 1,2,\ldots..14$); S = speci[illegible] = 1,2); T = time ($l = 1,2, \ldots 8$); E = error term ($m = 1,2,\ldots 160$). Flooding, species and time were considered fixed effects, bucket within treatment was a random effect.

Data were evaluated by analysis of variance using version 8.1 software (1996; SAS Institute Inc., Cary, NC) to determine the main and interactive effects of flooding, time and species. Means were compared for significant differences at $P < 0.05$ by Tukey's test.

3.2.3 Flooding experiment

3.2.3.1 Measurements of net assimilation (NA), stomatal conductance (g_s) & water use efficiency (WUE)

Net assimilation (NA), Stomatal conductance (g_s) and water use efficiency (WUE; net carbon assimilation rate divided by transpiration rate) of the flooded and non-flooded black spruce and tamarack seedlings were measured using an open-system infrared gas analyzer (IRGA) (LCA-3: Analytical Development Company Ltd. Hoddesdon, U.K.) equipped with a conifer cuvette (Macdonald and Lieffers 1990). Net assimilation and stomatal conductance rate were calculated as described by von Caemmerer and Farquhar (1981) and expressed on a leaf area basis. An artificial light source was used to supplement the PAR to 1000 μmol m^{-2} s^{-1}. The uppermost shoots of a randomly selected seedling from each species and treatment combination were placed in the cuvette for gas exchange measurements. Gas exchange measurements were taken before mid-day to avoid the midafternoon decline in the photosynthesis rates. Gas exchange and all other physiological measurements were taken on days 2, 5, 8, 11, 14, 18, 22, and 28 after flooding imposition and expressed on a leaf area basis. Needles were carefully detached from the stem and their surface areas measured by digitizing the scanned images (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA).

3.2.3.2 Root hydraulic conductance:

Root hydraulic conductance (K_r) is defined as water flow rate (kg s^{-1}) per unit pressure drop (MPa) driving flow through the entire root system whereas root hydraulic conductivity (L_p) is obtained by dividing the K_r value by root volume (measured by the water displacement method) and expressed in kg MPa $^{-1}$ s $^{-1}$ cm $^{-3}$ root volume. Root hydraulic conductance (K_r) was measured in intact roots of the same seedlings used for the gas exchange measurements, using a High Pressure Flow Meter [HPFM] (Dynamax, Houston, TX, USA) as described by Tyree et al. (1995). The use of HPFM allows for measurements of intact roots since the water is applied under increasing pressure through an excised stem (around the root collar level) into the root system (Tyree et al. 1995).

In this experiment, the stems of both species were cut at 2 cm above the root collar and flow rates of all the seedlings were measured at same temperature over a range of pressure from 0 to 2.75 MPa to obtain a linear pressure – flow relationship Tyree et al. (1995). Root hydraulic conductance of five root systems (from the same seedlings where all other gas exchange measurements were done) was measured from each species x treatment combination on each measurement day and was expressed in kg MPa $^{-1}$s^{-1}.

3.2.3.3 Electrolyte leakage

Following measurements of hydraulic conductance, electrolyte leakage, (a measure of the cell integrity and cell membrane leakiness) was measured on the same seedlings using a conductivity meter HI 8733 (Hanna Instruments Inc., Woonsocket, RI, USA) as described by Zwiazek and Blake (1990) and Renault et al. (1998). Needles (approximately 0.5 g) were taken from five seedlings per treatment combination (black spruce and tamarack, flooded and non-flooded), washed with deionized water three times and placed in separate tubes, each containing 20-ml of deionized water. After 5 hours of incubation on an orbital shaker at 50 rpm, initial measurements were taken for electrical conductivity of the solution. Total electrolytes of the samples were obtained by autoclaving the samples at 121^{0}C for 15 minutes followed by freezing overnight at –85^{0}C. The samples were thawed by placing them in a water bath at room temperature for about 5 hours. Total electrolytes of the sample solution were then measured and electrolyte leakage was calculated as a percentage of the total electrolytes.

3.2.3.4 Ethylene evolution

Finally, ethylene evolution was determined for the same seedlings using a Hewlett Packard 5890 Series II gas chromatograph (Hewlett Packard, Missisauga, ON, Canada) equipped with a flame ionization detector (FID) and an electronic integrator. The separation was carried out on a 30-m-long, 0.31-mm internal diameter GS-Q column (J & W Scientific, Folson, CA, USA) under the following conditions: oven temperature 60^{0}C, injector and detector temperatures 150^{0}C. The carrier gas was helium with linear flow rate of 30 cm s^{-1}. This GS-Q column is capable of distinguishing between ethylene and ethane.

To measure ethylene evolution from both flooded and non-flooded black spruce and tamarack seedlings, root samples and needles were enclosed in 9-ml septum vials and incubated for 10h at room temperature (22±2°C) (modified after Tang and Kozlowski 1984*b*). A 0.025ml sample of the ethylene-containing air was injected into the gas chromatograph column. The concentration of ethylene was determined by comparing the peak area with pure ethylene standards. Released-ethylene was expressed as nL (g needle dry weight^{-1} h^{-1}.

3.2.4 Ethylene experiment

3.2.4.1 Treatment application and experimental design

Six-month-old black spruce and tamarack seedlings, grown as described above, were used in the experiment. Roots of an intact seedling were placed in 500-ml brown non-reactive plastic containers with half of the volume filled with half-strength Hoagland's solution (Kamaluddin and Zwiazek 2002). Each seedling root systems was supplied with 20 μL L^{-1} of ethylene from a pure ethylene gas cylinder through a 1-mm diameter tube that reached to the bottom of the container, which was then closed tightly. Control seedlings were not given any ethylene but were kept in sealed containers. After the root systems had been exposed to the entrapped ethylene for 12 h, a 25 μl syringe was used to withdraw a sample of the entrapped ethylene to determine its concentration by gas chromatography. The peak area of the sample was compared with that produced by standard (PRAXAIR, Danbury, CT, USA) pure ethylene. A 2X2 factorial design was used in the ethylene experiment with species (black spruce and tamarack) and ethylene addition (with or without) as main (fixed) effects. Twelve hours after application of the ethylene, gas exchange, L_p and needle electrolyte leakage were measured as described above. Data were evaluated by analysis of variance using SAS version 8.1 software (1987; SAS Institute).

3.2.4.2 Root respiration

Root respiration of the whole root system was also measured by recording oxygen uptake with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA). Root systems of the ethylene-treated and un-treated black spruce and tamarack seedlings (n = 5) were placed in an airtight cylinder containing half-strength Hoagland's solution. Oxygen uptake rates were recorded every two minutes for 20 minutes. Respiration rate was expressed as average of oxygen uptake over time expressed in mg l^{-1}.

3.3 Results

3.3.1. Flooding experiment

Root morphology:

Visible damage of roots was observed in flooded seedlings of both species. Flooding reduced the root mass in both flooded black spruce and tamarack seedlings compared to their non-flooded controls (not quantified, easily visible). No adventitious roots were observed in any flooded seedlings while stem hypertrophy (swelling at the flood-water line) was observed in flooded tamarack seedlings.

Physiological responses:

Flooding significantly reduced A_{net}, g_s and K_r and increased WUE and needle electrolyte leakage in both species. Flooding caused a significant (P <0.0001) reduction in A_{net} in both species compared with the non-flooded controls (Figure 3.1A, Table 3.1a). Non-flooded black spruce seedlings maintained a higher A_{net} per unit leaf area than non-flooded tamarack seedlings throughout the experiment. The same trend was observed in flooded seedlings until 14 days of flooding after which A_{net} of black spruce declined sharply. There were no significant effects of bucket within flooding treatment on the measured response variables.

A significant ($P < 0.0001$) reduction in g_s was also observed in both species after flooding (Figure 3.1B, Table 3.1a), but there was no significant difference between species. Flooding significantly ($P < 0.0075$) increased WUE in both species compared to their non-flooded controls (Figure. 3.1C, Table 3.1a).

Flooding significantly ($P < 0.0001$) reduced K_r in both species compared with their non-flooded controls (Figure 3.1D, Table 3.1a). However, on day 14, flooded tamarack seedlings were able to maintain a higher root hydraulic conductance compared with flooded black spruce seedlings.

Electrolyte leakage significantly increased ($P < 0.0001$) in the foliage of both black spruce and tamarack seedlings after 14 days of flooding compared with their non-flooded controls (Figure 3.1E, Table 3.1a). On day 22, electrolyte leakage was higher in flooded black spruce seedlings than in flooded tamarack seedlings. In flooded black spruce seedlings, needles appeared to develop necrosis on day 14 that continued to increase over time, and about 40% of the total needles showed signs of necrosis by the end of the experiment. The severity of needle necrosis in flooded black spruce seedlings paralleled the degree of electrolyte leakage over time. In contrast, no necrosis was observed in flooded tamarack seedlings.

Both flooded black spruce and tamarack seedlings (root and needle samples) produced ethylene. However, no significant trends in relation to species or flooding treatment were observed.

3.3.2 Ethylene treatment

Application of exogenous ethylene resulted in a significant reduction in A_{net} ($P < 0.0021$) in black spruce seedlings but not in tamarack seedlings (Figure 3.2A, Table 3.1b). Stomatal conductance declined significantly (P<0.0004) in both species after ethylene treatment (Figure 3.2B, Table 3.1b). Ethylene treatment also resulted in a significant reduction in root respiration ($P < 0.0001$) in both species (Figure 3.3A, Table 3.1b). The magnitude of these ethylene- induced reductions was higher in black spruce than in tamarack (Figures 3.2B and 3.3A). Root hydraulic conductivity increased significantly ($P < 0.0005$) in tamarack in response to ethylene treatment, but there was no significant effect for black spruce (Figure 3.3B, Table 3.1b). Ethylene treatment caused an increase in electrolyte leakage for both species, but the increases were statistically insignificant.

3.4 Discussion

Although the general patterns of response were similar for both species there were some key differences in timing and magnitude of responses. Overall, tamarack seedlings were more resistant to 4 weeks of flooding than black spruce seedlings. In both species, A_{net} and g_s were reduced from day 2 through day 28 of the flooding treatment. Stomata began to close within 2 days of flooding (Figure 3.1B). The functional significance of stomatal closure seems to be to restrict shoot water loss during times of lowered K_r, an effect observed simultaneously in both black spruce and tamarack from day 2 of flooding imposition (Figure 3.1D).

Early stomatal closure during flooding can occur with and without leaf dehydration (Kramer and Jackson 1954, Pereira and Kozlowski 1977). For example, a reduction in soil oxygen can also induce stomatal closure without a change in leaf water potential (Sojka and Stolzy 1980, Stolzy et al. 1961). However, Else et al. (2001) reported that stomatal closure and slower leaf expansion in flooded *Ricinus communis* were caused by a decrease in L_p that reduced leaf water potential. Stomatal control mechanisms in flooded plants may vary with duration of flooding, condition of the root system, and growth stage (Coutts 1981). Stomata of *Picea sitchensis* (Bong.) Carriere seedlings closed gradually as shoots dehydrated during a period of flooding (Coutts 1981). We saw a similar trend of gradual stomatal closure coupled with a reduction in K_r, presumably caused by the impact of oxygen deficits on membranes in black spruce and tamarack. Our results contrast with those of Reece and Riha (1991) who reported that 10 days of flooding had no effect on K_r of tamarack and white spruce.

Although K_r and g_s were significantly reduced in our flooded seedlings, there was an increase in WUE on day 14 of flooding. This increase in WUE corresponds to a gradual decline in g_s, which is likely associated with the fact that the flow of water vapor through the stomata is more sensitive to partial closure than the flow of carbon dioxide. An increase in electrolyte leakages in flooded black spruce seedlings is probably a result of membrane damage. As a consequence of membrane damage, A_{net} declined in flooded black spruce seedlings. Although flooded black spruce seedlings were able to maintain higher A_{net} and g_s than flooded tamarack seedlings during the initial phase of flooding, they showed reduced K_r. Black spruce needles also exhibited discoloration and necrosis, perhaps reflecting membrane damage. In needles of flooded Norway spruce and Scots pine, ultrastructural symptoms appear to be related to the development of nutrient imbalance (Palomaki et al. 1994). In contrast to flooded black spruce seedling, flooded tamarack seedlings had reduced A_{net} and g_s, but were able to maintain higher K_r. Consequently flooded tamarack suffered little membrane damage and showed no visual symptoms of damage.

Flooded black spruce seedlings did not exhibit any morphological acclimation to flooding, whereas tamarack showed stem hypertrophy (swelling at the flood-water line). Stem hypertrophy is reported to facilitate the aeration of submerged roots (Vartapetian and Jackson 1997). Blake and Reid (1981) reported that hypertrophy, especially the production of spongy aerenchyma tissue above the water level, may allow the plant to eliminate ethylene as well as aerate the roots; therefore, this mechanism could help the plant avoid flooding stress. We did not observe adventitious roots on any flooded seedlings.

Compared with black spruce, tamarack had a higher root mass with many new roots that were maintained even during flooding. This is of particular importance since young roots and root tips have the highest respiration rate per unit volume of tissue (Nilsen and Orcutt 1996). It has previously been reported that tamarack can sustain root respiration during anaerobiosis (Conlin and Lieffers 1993). It should also be noted that, during flooding, nitrate decreases and soil nitrogen is dominated by ammonium ions (Armstrong et al. 1994). Because uptake of ammonium requires root respiration (Chapin et al. 1987), the ability of tamarack to maintain root respiration during anaerobiosis might also contribute to its ability to withstand prolonged flooding.

In many plants, increased ethylene synthesis is associated with oxygen deficiency and flooding (Kawase 1976, Wample and Reid 1979, Drew et al. 1981, Metraux and Kende 1983, Raskin and Kende 1984). Ethylene concentration in shoots is often increased when roots are submerged in water of low oxygen content (Kawase 1972, Jackson and Campbell 1976). Membrane damage and leaf epinasty are often attributed to increased ethylene production in plants subjected to stress (Blake and Reid 1981). In our study, electrolyte leakage increased significantly in flooded seedlings of both black spruce and tamarack (Figure 1E); however, we did not observe a consistent trend in ethylene accumulation in flooded black spruce and tamarack seedlings.

Because exogenous ethylene produces symptoms typical of flooding in plants [e.g., formation of adventitious roots, leaf epinasty, and stem hypertrophy (Kawase 1972, Wample and Reid 1979)], seedlings of both black spruce and tamarack were treated with exogenous ethylene. We note that the root containers were sealed during the ethylene treatment, so the observed response may be a result of the combined effects of ethylene, reduced oxygen concentration and high carbon dioxide.

The reductions in A_{net} and g_s in response to the exogenous ethylene treatment are consistent with earlier findings (Govindrajan and Poovaiah 1982, Pallas and Kays 1982). The effect of ethylene on g_s generally exceeds its effect on A_{net}, although the ethylene-induced changes in g_s and A_{net} are not necessarily well coupled in some species (Taylor and Gunderson 1986). In our study, black spruce and tamarack differed in their responses to ethylene application.

Root hydraulic conductivity was measured to account for the variability of root volume among seedlings because the duration of the ethylene treatment was short. Increased hydraulic conductivity in ethylene-treated seedlings of both black spruce and tamarack might be explained by the increased activity of aquaporins. In plants, aquaporins are thought to regulate the water flow through membranes during growth, development and stress responses (Cheng et al. 1997). Evidence suggests that ethylene enhances protein phosphorylation (Raz and Fluhr 1993), which in turn enhances aquaporin activity and increased hydraulic conductance. Exogenous ethylene also increased L_p in hypoxic aspen (Kamaluddin and Zwiazek 2002). Increased K_r may be a key factor underlying the enhanced growth performance and higher net photosynthesis of *Coffea canephora* Pierre ex Froehn. plants under conditions of excess water (Fahl et al. 2001).

Continued water uptake by roots is oxygen dependent because both the active ion uptake needed to maintain an osmotic gradient across the root (Rowe and Beardsell 1973) as well as membrane hydraulic conductivity (Glinka and Reinhold 1962) are known to require oxygen. Although ethylene treatment reduced root respiration in both species, the magnitude of the reduction was greater in black spruce, which may account for this species' lower L_p compared with tamarack.

In conclusion, we demonstrated that tamarack exhibits greater flooding resistance compared with black spruce. Black spruce was more responsive to exogenous ethylene than tamarack. We suggest that morphological acclimations, maintaining high rates of K_r and the ability to withstand flooding-induced ethylene accumulation are the key factors conferring on tamarack greater flooding tolerance than that possessed by black spruce.

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Table 3. 1. Analysis of variance (ANOVA) results for different dependant variables in response to flooding showing the levels of significance (*P*) for the main fixed effect flooding, flooding x time and flooding x species (a), and for the main effect of ethylene and ethylene x species (b).

(a) Flooding treatment:

Variables	Flooding	Flooding x Time	Flooding x Species
Net assimilation	0.0001	0.0001	0.0001
Stomatal conductance	0.0001	0.0001	0.3300
Water use efficiency	0.0075	0.3677	0.7026
Root hydraulic conductance	0.0001	0.3893	0.0014
Electrolyte leakage	0.0001	0.0001	0.7431

(b) Ethylene treatment:

Variables	Ethylene	Ethylene x Species
Net assimilation	0.0021	0.0645
Stomatal conductance	0.0004	0.8098
Root respiration	<0.0001	0.0221
Root hydraulic conductivity	0.0005	0.0507

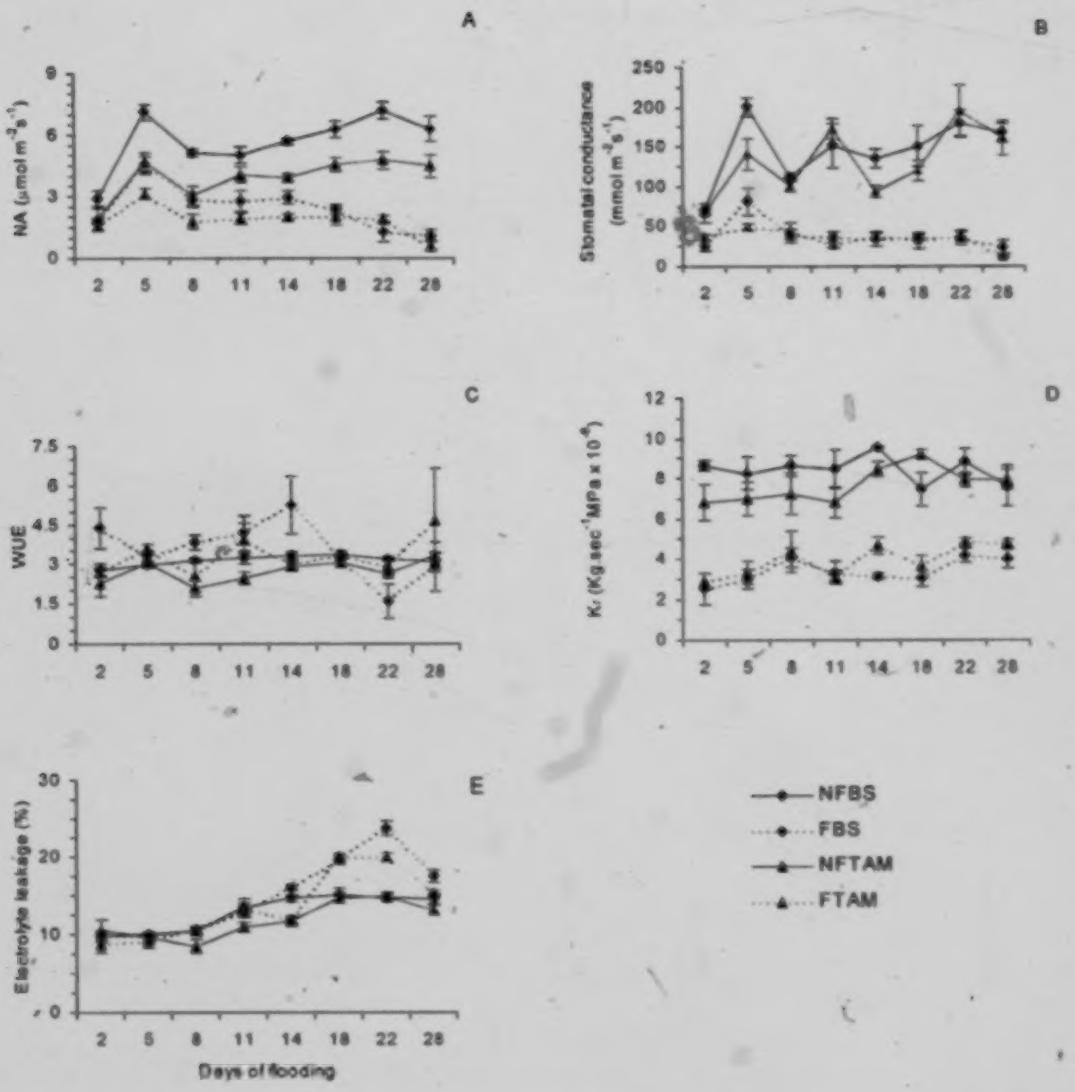

Figure 3.1. Effects of flooding for 28 days on net assimilation (A), stomatal conductance (B), water use efficiency (C), root hydraulic conductance (D), and electrolyte leakage (E) in black spruce and tamarack seedlings. NFBS = non-flooded black spruce, FBS = flooded black spruce, NFTAM = non-flooded tamarack, and FTAM = flooded tamarack. Vertical bars indicate s.e. (n=5).

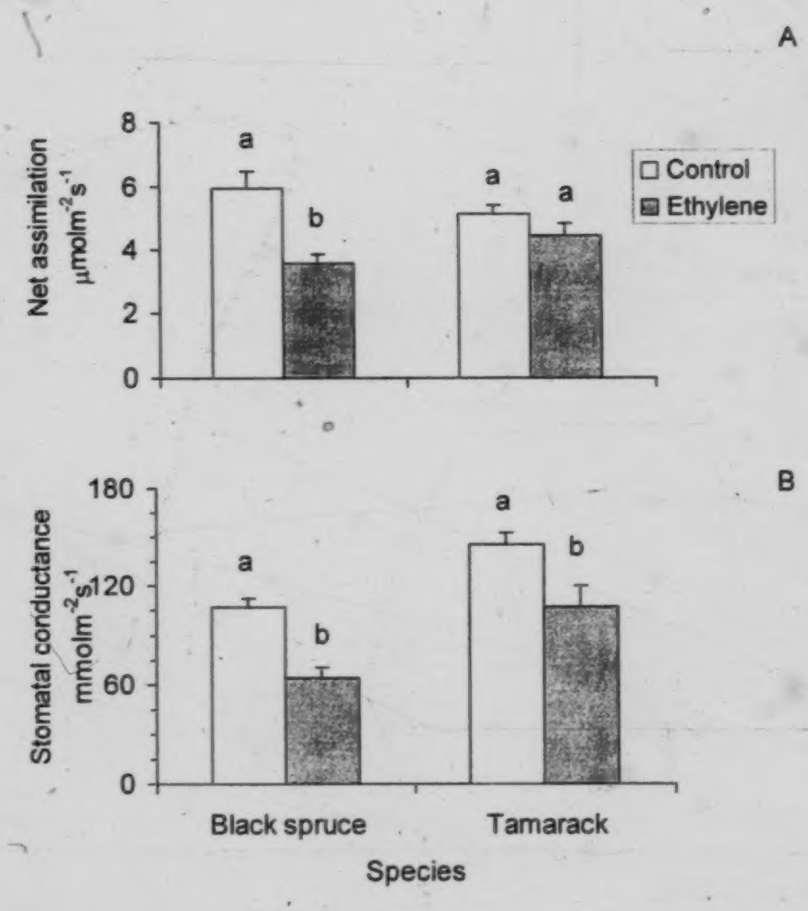

Figure 3.2. Effect of exogenous ethylene on net assimilation (A), and stomatal conductance (B) in black spruce and tamarack seedlings. Vertical bars indicate s.e. (n=5). Bars with the same letter are not significantly different at $P < 0.05$.

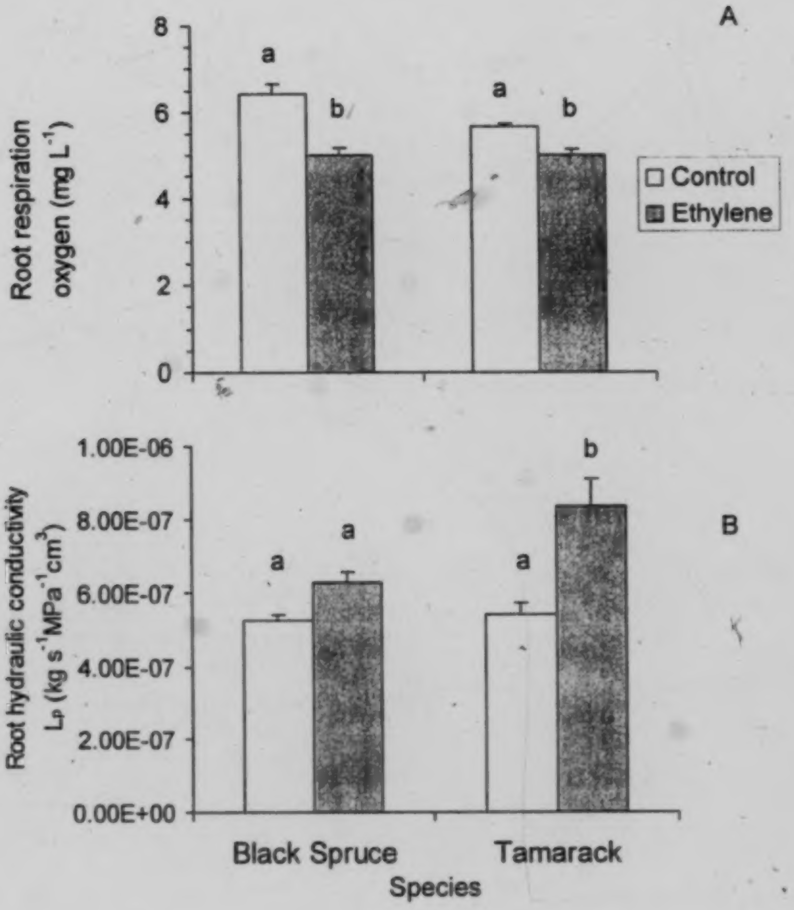

Figure 3.3. Effect of exogenous ethylene on root respiration (A), and root hydraulic conductivity (B) in black spruce and tamarack seedlings. Vertical bars indicate s.e. (n=5). Bars with the same letter are not significantly different at $P < 0.05$.

CHAPTER FOUR[*]

Ecophysiological adaptations of black spruce (*Picea mariana*) and tamarack (*Larix laricina*) seedlings to flooding.

4.1 Introduction

Low oxygen concentration in the rooting medium due to waterlogging reduces root and shoot growth of trees by affecting many plant physiological processes, including net assimilation, transpiration, stomatal conductance (Anderson et al. 1984; Levan and Riha 1986; Pereira and Kozlowski 1977; Pezeshki and Chambers 1986), carbohydrate metabolism and respiratory activity (Pezeshki 1994).

Flooding triggers ethylene accumulation in plants (Tang and Kozlowski 1984a and b; Blake and Reid 1981). Evidence suggests that in some species morphological responses to flooding, such as hypertrophy of lenticels and formation of adventitious roots are associated with increased production of ethylene (Blake and Reid 1981). The close association of adventitious root formation and stomatal reopening in some species suggests that these aerenchymatous roots may play a key role in the recovery of gas exchange during flooding stress (Tang and Kozlowski 1984*a*). In addition, flood-induced adventitious roots increase water absorption for flooded plants (Jackson and Drew 1984; Tsukahara and Kozlowski 1985).

Root respiration has been shown to decline as a result of hypoxia in wheat (Huang and Johnson 1995), and aspen (Kamaluddin and Zwiazek 2002). Carbohydrate concentrations may remain the same or increase in roots and shoots under hypoxic conditions (Huang 1995). The rate and extent of increase in sugar content depends on the environmental conditions, species and even on genotypes within the same species (Setter et al. 1987). Soluble carbohydrates such as sucrose, glucose or fructose are of particular importance because of their direct relationship with respiration. Thus, carbohydrate allocation patterns appear to be critical for hypoxia tolerance (Huang and Johnson 1995).

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[*] A version of this chapter has been accepted. M. Anisul Islam and S. Ellen Macdonald 2003. Ecophysiological adaptations of black spruce (*Picea mariana*) and tamarack (*Larix laricina*) seedlings to flooding. Trees: Structure and Function

Two conifers, black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch), form open forest stands on peatlands in Alberta, Canada (Lieffers and Macdonald 1990). These organic sites have very low productivity and are characterized by a high water table (Larsen 1982), poor soil aeration (Campbell 1980) and undergo significant variation in depth of water table during the growing season. Oxygen supply to tree roots decreases significantly when the water table rises close to the peat surface (Kozlowski 1984; Mannerkoski 1985).

Although black spruce and tamarack co-occur on moderately minerotrophic peatlands, tamarack tends to dominate on wetter, minerotrophic sites (Jeglum and He 1996; Kenkel 1987; Tilton 1977). Black spruce and tamarack possess contrasting growth habits with the former one being evergreen with sclerophyllous leaves, while the latter is deciduous with sclerophyllous leaves (Tyrell and Boerner 1987). Black spruce exhibits fixed growth i.e., its growth is determined by the process of bud set in the previous fall. Tamarack has determinate growth of short shoots but also possesses long shoots displaying indeterminate growth, thus allowing a greater degree of variation in annual growth in response to conditions during the growing season (Clausen and Kozlowski 1967).

In a previous study (Islam et al. 2002), we have shown that flooded tamarack seedlings were able to maintain higher root hydraulic conductance compared to flooded black spruce seedlings and had no visible flooding damage. However, the possible role of stored carbohydrate and root respiration on root hydraulic conductance was not investigated. Also, since the seedlings were only six-months old, the differences in response that could occur from determinate and indeterminate growth habit in these two species, could not be examined. In the present study, we examined the effect of 34 days of soil flooding on gas exchange, root hydraulic conductance and membrane damage of black spruce and tamarack and explored the relationships of these parameters with carbohydrate metabolism and root respiratory activity in these two species at an age when determinate and indeterminate growth is in effect after winter hardening. We hypothesized that flooding tolerance in tamarack is conferred by maintenance of root water uptake and respiration-dependent processes.

4.2 Materials and Methods

4.2.1. Plant material and growing conditions

Eighteen-month-old black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) seedlings were used in the flooding experiment. Seeds were obtained from the Alberta Tree Improvement and Seed Center (ATISC acc #4042; see also section 3.2.1). Seeds were stratified for three weeks at 4^0C and then germinated in Petri-dishes. One week after germination, seedlings were transplanted to Spencer-Lemaire (300ml) containers (Spencer-Lemaire Industries Ltd. Edmonton, AB. Canada) containing planting medium Pro-Mix BX (1:1:1; peat:moss:vermiculite; Premier Horticulture Inc., Riviere-du-Loup, Que. Canada) and placed in a growth chamber with

21°C/18°C day/night temperature, 65% relative humidity and 16-h photoperiod with photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} provided by fluorescent lamps. Seedlings were watered to run off every other day and fertilized once a week with 30-10-10 N-P-K fertilizer (Plant-prod®- Plant Products Co. Ltd., ON, Canada).

After 24 weeks of growth, we gradually reduced the growth chamber temperature and photoperiod in order to induce winter hardening. Plants were then placed in a cold room with 10⁰C temperature and 10 h photoperiod for six weeks then in 4⁰C and 8 h photoperiod for four months. At this stage, tamarack seedlings had shed their needles. After that seedlings were brought back to 15⁰C temperature and 10 h photoperiod for two weeks. Thereafter, seedlings were transplanted to 12.7 cm pots with Pro-Mix BX (as above) and returned to the original growing conditions (21°C/18°C day/night temperature, 65% relative humidity and 16-h photoperiod) and were grown for 26 weeks until application of the treatment.

4.2.2. Treatment application and experimental design

Treatment application commenced when the seedlings flushed and were actively growing. Seedlings from both black spruce and tamarack were randomly placed in buckets where flooding was imposed by submerging the seedlings to root-collar level. Perforated buckets were used for control (non-flooded) seedlings to ensure adequate drainage. There were seven buckets for each treatment and twelve seedlings (six from each species) were randomly assigned to each bucket. Data were collected on day 2, 5, 8, 12, 16, 21, 27 and 34 after treatment imposition. On each measurement day, different physiological parameters were measured on five randomly selected seedlings from each species x treatment combination (no more than one of a given species from a given bucket). A total of 160 seedlings (N) were used in this experiment. The experiment was a split-split-plot design (between subjects: flooding, within-subjects: species and time) and buckets were considered nested within treatments.

The statistical model is as follows:

$$Y_{ijklm} = \mu + F_i + B_{j(i)} + S_k + FS_{ik} + BS_{j(ik)} + T_l + TF_{il} + TS_{kl} + TFS_{ikl} + \varepsilon_{ijklm}$$

Where, Y_{ijklm} = Physiological parameter (e.g., net assimilation, stomatal conductance, electrolyte leakage etc.), μ = overall mean, F = flooding treatment (i = 1,2), B = bucket within treatment (j = 1,2,.....7); S = species (k = 1,2); T = time (l = 1,2, ...8); ε = error term (m = 1,2,...160).

Data were analyzed using analysis of variance (using SAS version 8.1; SAS Institute Inc., Cary, NC. 1996) to determine the main and interactive effects of flooding, time and species. Means were compared for significant differences at $P < 0.05$ using Tukey's test.

4.2.3. Measurements

4.2.3.1. Gas exchange

Net assimilation (A_{net}), stomatal conductance (g_s) and water use efficiency (WUE; net carbon assimilation rate divided by transpiration rate) of the flooded and non-flooded black spruce and tamarack seedlings were measured using an infrared gas analyzer (CIRAS 1: PP Systems, Haverhill, MA, USA) equipped with a automatic conifer cuvette (PLC(C), PP Systems Inc.). Leaf temperature was maintained at 20^{0}C and relative humidity at 40%. The flow rate was 250 ml min^{-1} and light level was maintained at 1050

μmol m^{-2} s^{-1}. Net assimilation and stomatal conductance rates were expressed on a leaf area basis. The uppermost shoots of a randomly selected seedling from each species and treatment combination were placed in the cuvette for gas exchange measurements. Gas exchange measurements were taken before mid-day to avoid the midafternoon decline in the photosynthesis rates. Gas exchange and all other physiological measurements (except root respiration and carbohydrate concentration) were taken on days 2, 5, 8, 12, 16, 21, 27, and 34 after flooding imposition. Needles were carefully detached from the stem and their surface areas were measured after computer scanning (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA).

4.2.3.2. Root hydraulic conductance

Root hydraulic conductance (K_r) is defined as water flow rate (kg s^{-1}) per unit pressure drop (MPa) driving flow through the entire root system, whereas root hydraulic conductivity (L_p) is obtained by dividing the K_r value by root volume and expressed in kg MPa^{-1} s^{-1} cm^{-3} root volume. Root hydraulic conductance (K_r) was measured on intact roots using a High Pressure Flow Meter [HPFM] (Dynamax, Houston, TX, USA) as described by Tyree et al. (1995). The use of a HPFM allows for measurements of intact roots since the water is applied under increasing pressure through an excised stem (around the root collar level) into the root system (Tyree et al. 1995). In this experiment, stems of seedlings were cut following gas exchange measurements two cm above the root collar and flow rates were measured over a range of pressures from 0 to 2.75 MPa to obtain a linear pressure – flow relationship (Tyree et al.1995). Root hydraulic conductance of five root systems was measured from each species x treatment combination on each measurement day and was expressed in kg MPa $^{-1}$s^{-1}. Root hydraulic conductivity of adventitious roots and similarly sized flooded tamarack roots (n = 4) was also measured. Adventitious roots and flooded tamarack roots with a length of 5.0 cm or higher and a diameter of 0.8 mm or greater were used for measurement since this was the minimum diameter that could fit in with the HPFM. Individual root volume was calculated by determining the radius and length of the roots. A student's t-test was performed to compare the means.

4.2.3.3. Electrolyte leakage

Electrolyte leakage, a measure of cell integrity and of cell membrane leakiness, was measured using a conductivity meter (HI 8733 Hanna Instruments Inc., Woonsocket, RI, USA) as described by Zwiazek and Blake (1990) and Renault et al. (1998). Needles (approximately 0.5 g) were taken from five seedlings per species x treatment combination (after gas exchange and root hydraulic conductance measurements), washed with deionized water three times and placed in separate tubes, each containing 20-ml of deionized water. After 5 hours of incubation on an orbital shaker at 50 rpm, initial measurements were taken for electrical conductivity of the solution. Total electrolytes of the samples were obtained by autoclaving the samples at 121^{0}C for 15 minutes followed by freezing overnight at –85^{0}C. The samples were thawed by placing them in a water bath at room temperature for about 5 hours. Total electrolytes of the sample solution were then measured and electrolyte leakage was calculated as a percentage of the total electrolytes.

4.2.3.4. Root respiration

Root respiration of the whole root system was measured by recording oxygen uptake with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH). Root respiration was measured 2, 8, 16 and 27 days after flooding treatment was imposed.. Root systems of black spruce and tamarack seedlings (n = 5 per species x treatment combination) were placed in an airtight cylinder containing half-strength Hoagland's solution (Kamaluddin and Zwiazek 2002). Oxygen uptake rates were recorded every two minutes for 20 minutes. Respiration rate was expressed as average of oxygen uptake over time expressed in mmol L^{-1}.

4.2.3.5. Carbohydrate analysis

Carbohydrates were extracted three times with hot 85% ethanol at 95^0C from a sample portion (approximately 0.05g dry wt.) of the shoots and roots of flooded and non-flooded black spruce and tamarack seedlings (n=5 per species x treatment combination). Carbohydrate concentrations were measured from both flooded and non-flooded black spruce and tamarack seedlings 2, 8, 16 and 27 days after the treatment application. Soluble carbohydrate (sugar) concentrations were determined colorimetrically using phenolsulfuric acid as described by Smith et al. (1964). Starch was extracted from the same plant samples as soluble carbohydrate with 2.0mL of 0.1N NaOH and hydrolyzed by an enzyme mixture containing 0.5mL of alpha-amylase (EC 3.2.1.1, ICN 190151, from *Bacillus licheniformis*) and amyloglucosidase (EC 3.2.1.3, Sigma A3514, from *Aspergillus niger*) and incubated for 41 h before colorimetric measurements with the glucose-oxidase/peroxidase-o-dianisidine reagent (Sigma Glucose Diagnostic Kit 510A)

(Haisig and Dickson 1979). Absorbance readings were determined with an Ultrascope III spectrophotometer (Pharmacia LKB, UK) and sugar and starch concentrations were calculated on a dry weight basis.

4.3. Results

4.3.1. Morphological responses

Visible damage of roots was observed in flooded seedlings of both species. Flooding reduced the root mass in both black spruce and tamarack seedlings compared to their non-flooded controls (not quantified, easily visible). Flooded tamarack seedlings developed adventitious roots beginning 16 days after the imposition of flooding treatment. After this point in time, 86% of the flooded tamarack seedlings had adventitious roots (average of 13 adventitious roots per seedling with an average length of 15 mm). Flooded black spruce lacked any such morphological acclimation. Needle chlorosis and necrotic needle tips were observed only in flooded black spruce seedlings.

4.3.2. Physiological responses

Flooding significantly reduced net assimilation, stomatal conductance, root hydraulic conductance and root respiration while increasing needle electrolyte leakage in both species. Flooding also increased sugar concentration in the shoot while reducing it in the roots in both species. A significant ($P < 0.0001$) reduction in net assimilation (A_{net}) rate was observed in both black spruce and tamarack seedlings under flooding compared to their non-flooded controls (Figure 4.1a; Table 4.1). Flooded tamarack seedlings maintained significantly ($P < 0.0001$) higher A_{net} than flooded black spruce seedlings on day 8, 12, 16, 27 and 34 during the experiment (Figure 4.1a). Of all the parameters measured, significant effects of buckets within treatments were observed only in net photosynthesis and root sugars (Table 4.1).

A significant ($P < 0.0001$) reduction in stomatal conductance was also observed in both species under flooded conditions compared to non-flooded seedlings (Figure 4.1b). Stomatal conductance declined from day 2 in flooded black spruce and tamarack seedlings. At the end of the experiment, flooded black spruce had a stomatal conductance value of 20.08 mmol m^{-2} s^{1} compared to 73.85 mmol m^{-2} s^{-1} maintained by flooded tamarack. Flooded tamarack seedlings maintained significantly ($P < 0.0001$) higher stomatal conductance than flooded black seedlings throughout the experiment (Figure 4.1b; Table 4.1).

Flooding increased water use efficiency (WUE) in tamarack but decreased it in black spruce as compared to non-flooded seedlings (Figure 4.1c; Table 4.1). Flooding significantly ($P < 0.0263$) reduced root hydraulic conductance compared to non-flooded controls (Figure 4.2a). Although there were significant species ($P < 0.0001$), time ($P < 0.0209$), and species*time ($P<0.0002$) effects, the interaction of treatment*species*time was not significant. Flooded tamarack had significantly higher root hydraulic conductance than flooded black spruce seedlings on days 27 and 34. Hydraulic conductivity of adventitious roots was significantly ($P<0.0001$) higher than the conductivity in similarly sized flooded tamarack roots (Figure 4.2b).

Electrolyte leakage increased significantly in both flooded black spruce and tamarack seedlings (Figure 4.3a; Table 4.1). Overall there was no significant species effect but flooded black spruce had significantly higher leakage than flooded tamarack on day 16 and 34 suggesting a significant species*treatment effect. There were also significant species*treatment, treatment*time, species*time and treatment*species*time effects.

Root respiration rate was significantly reduced (P <0.0001) in both species under flooding (Figure 4.3b). However, flooded tamarack maintained higher root respiration than flooded black spruce seedlings throughout the experiment. Although the species*treatment interaction was not significant but the three way interaction of treatment*species*time was significant (Table 4.1).

Sugar content in shoots of flooded and non-flooded seedlings was not significantly different until day 16, then increased significantly in flooded seedlings of both species compared to the non-flooded controls (Figure 4.4a). Root sugar content increased initially but declined in flooded seedlings of both species after day 16. Root sugars in flooded tamarack increased earlier than flooded black spruce and declined when adventitious roots emerged. Starch content in shoots of flooded black spruce remained the same as non-flooded seedlings until day 8 and then increased drastically compared to their non-flooded controls while no such trend was observed in flooded tamarack seedlings (Figure 4.5a). However, root starch content declined in both species (Figure 4.5b) but not significantly. There was significant species difference in root starch concentration (Table 4.1).

4.4. Discussion

The results of our study suggest that tamarack was relatively more tolerant to flooding than black spruce, in agreement with the results of our previous study (Islam et al. 2002). For both six-month old (Islam et al. 2002) and 18-month old (this study) seedlings, net assimilation and stomatal conductance were reduced during flooding in both species. However, the magnitude of reduction we observed in 18-month old seedlings was much less than for six-month old seedlings of both species (Islam et al. 2002).

The early reduction in the rate of photosynthesis of flooded plants is correlated with stomatal closure, resulting in decreased CO_2 absorption by leaves (Pezeshki et al. 1996). Subsequently, photosynthetic processes may be adversely affected by the metabolic consequences of prolonged hypoxia (Bradford 1983a, 1983b). Flooding can also reduce net photosynthesis by reducing the amount or activity of carboxylation enzymes (Vu and Yelenosky 1991; Vu and Yelenosky 1992), by disruption of photosynthate transport, alteration in source-sink relationships, or reduced sink demand (Wample and Thornton 1984, Drew 1990).

As in our previous study (Islam et al. 2002), we observed a gradual reduction in stomatal conductance as well as root hydraulic conductance in both species, presumably due to the impact of oxygen deficit on membranes (Sojka and Stolzy 1980). Reduction in stomatal conductance in conjunction with reduced carbon assimilation during flooding has been observed in other woody plants (Davies and Flore 1986, Pezeshki et al. 1996). However, a reduction in root hydraulic conductivity coupled with reduced leaf water potential was the key factor for stomatal closure and slower leaf expansion in flooded *Ricinus communis* (Else et al. 2001). Similar reductions in g_s and K_r were observed in flooded *Pyrus* species (Anderson et al. 1984) and *Vaccinium corymbosum* (Davies and Flore 1986). Wan et al. (1999) have also shown a strong positive correlation between root hydraulic conductance and stomatal conductance and transpiration rates. In the present study (with 18-month old seedlings), differences in root hydraulic conductance between flooded tamarack and black spruce were much greater than for 6-month-old flooded seedlings of both species (Islam et al. 2002). Our results (with 18-month-old seedlings) contrast with those of Reece and Riha (1991) in which root hydraulic conductance of tamarack and white spruce (2-years-old) appeared to be unaffected after 10 days of flooding.

Although there was a gradual decline in stomatal conductance in flooded tamarack seedlings, there were apparent increases in root hydraulic conductance and WUE after 16 days in those seedlings, whereas both root hydraulic conductance and WUE were significantly lower in flooded black spruce seedlings. The increase of root hydraulic conductance in flooded tamarack seedlings coincided with the emergence of adventitious roots in those seedlings 16 days after flooding treatment began. Flooded black spruce seedlings did not have any adventitious roots. Morphological adaptations such as production of hypertrophied lenticels, aerenchyma tissue and adventitious roots have been attributed to the high nutrient-uptake efficiency of flood-tolerant species (Kozlowski 1997). It is reported that flood-induced adventitious roots increased water absorption by roots during flooding (Hook and Scholtens 1978, Jackson and Drew 1984, Tsukahara and Kozlowski 1985). Our results suggest that the emergence of adventitious roots, which had increased hydraulic conductivity, increased total root hydraulic conductance in flooded tamarack seedlings. Six-month-old flooded tamarack seedlings developed hypertrophied lenticels but lacked the emergence of adventitious roots (Islam et al. 2002).

Needle chlorosis and development of necrotic needle tips in flooded black spruce seedlings were possibly due to significantly higher membrane damage (i.e., increased needle electrolyte leakage). Needle chlorosis in black spruce might also have been caused by a reduced supply of nutrients, which has also been observed in some flooded woody plants (Larson et al. 1992; Smith and Bourne 1989). Higher membrane damage could also explain the drastic reduction in NA in flooded black spruce seedlings. In contrast, flooded tamarack seedlings showed less membrane damage and no visual symptoms of flooding damage.

Root respiration rates were reduced for both species under flooding, with a greater reduction for black spruce over the entire experiment. A reduction in root respiration was also observed in a hypoxia sensitive *Triticum aesivum* cultivar (Huang and Johnson 1995). Although it has been suggested that a low oxygen consumption rate can contribute to greater tolerance of hypoxia (Huang and Johnson 1995), our results contrast with that. Continued water uptake by roots is oxygen dependent because both the active ion uptake needed to maintain an osmotic gradient across the root (Rowe and Beardsell 1973) as well as membrane hydraulic conductivity (Glinka and Reinhold 1962) are known to require oxygen. Although both flooded black spruce and tamarack maintained low oxygen consumption in the present study, black spruce failed to maintain the higher root hydraulic conductance, which was observed in flooded tamarack.

Carbohydrates are the primary energy storage compounds in plants and their allocation pattern and translocation rate appear to be critical for hypoxia tolerance (Huang and Johnson 1995). The high accumulation of carbohydrates in shoots, compared to roots, in our study suggests that sufficient carbohydrates were available to support metabolic activity but root zone hypoxia prevented their export to the roots. Sugar accumulation in the shoots of both species during flooding was the same but root sugar was significantly higher in tamarack suggesting a continuous transport to the roots. Since tamarack could maintain indeterminate growth, it could potentially allocate carbon for the growth of shoot and root equally. The high concentration of carbohydrates might have contributed to the production of adventitious roots during flooding since production of adventitious roots is positively correlated with carbohydrate content (Veierskov 1988). However, it seems that black spruce having a determinate growth and being a slow growing species was unable to direct enough carbon to the roots. The high root sugar concentration in flooded tamarack might have contributed to the maintenance of their higher root respiration. Higher accumulation of carbohydrates was also observed in shoots of hypoxic *Atriplex amnicola* (Galloway and Davidson 1993). A reduction in the translocation of assimilates in hypoxic plants (Schumacher and Smucker 1985) leads to low carbohydrate availability in roots (Webb and Armstrong 1983). Oxygen deficient conditions inhibit root elongation due to low carbohydrate reserves in the root tips (Webb and Armstrong 1983). Barclay and Crawford (1983) have shown that tolerance of plants to flooding is dependent on the available carbohydrate reserves in the roots. In our study, although root sugars declined in both species, tamarack maintained higher sugar content throughout the experiment. Moreover, higher root respiration and root sugar content might have contributed to the higher root hydraulic conductivity through maintaining an osmotic gradient and facilitating active water uptake in flooded tamarack seedlings.

In conclusion, our study demonstrated that tamarack seedlings exhibit greater flooding tolerance than black spruce seedlings. We suggest that membrane function and respiration-dependent processes were responsible for the reduction in root hydraulic conductance in flooded plants. However, emergence of adventitious roots in flooded tamarack improved root water uptake. Morphological acclimations, maintaining high rates of root hydraulic conductance and respiration are likely the key processes that enable tamarack to withstand flooding effects better than black spruce.

4.5. References

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Table 4. 1. Results of Analysis of Variance (ANOVA) testing for main effects of flooding (Flood), Species (Spp), Time, and their interactions on net assimilation (A_{net}), stomatal conductance (g_s), water use efficiency (WUE), root hydraulic conductance (K_r), electrolyte leakage, root respiration, shoot sugars, root sugars, shoot starch and root starch of flooded and non-flooded black spruce and tamarack seedlings (n =5). Time, Flood*time, Spp*time, and Flood*spp*time used residual error as an error term.

	Response variables									
Source	NA	g_s	WUE	K_r	Electrolyte Leakage	Root respiration	Shoot sugars	Root sugars	Shoot starch	Root starch
Flood	<0.0001	<0.0001	0.0010	0.0263	<0.0001	<0.0001	<0.0001	0.7937	0.0053	0.1368
Buc(flood) (Error 1)	0.0022	0.1975	0.8200	0.3989	0.9732	0.2991	0.1885	0.0287	0.5213	0.2882
Spp	<0.0001	<0.0001	0.0184	<0.0001	0.5166	<0.0001	0.0018	<0.0001	0.0002	<0.0001
Spp*flood	0.1234	0.6372	0.0398	0.6246	<0.0001	0.2828	0.1238	<0.0001	0.1469	0.4695
Buc(Spp*flood) (Error 2)	0.0341	0.2726	0.5530	0.6705	0.5424	0.2064	0.6725	0.2360	0.0687	0.7219
Time	<0.0001	<0.0001	<0.0001	0.0209	<0.0001	<0.0001	<0.0001	0.0014	0.0097	0.0339
Flood*time	<0.0001	<0.0001	0.2807	0.0075	<0.0001	0.9647	0.0019	<0.0001	0.0784	0.6052
Spp*time	0.0313	0.6888	0.0085	0.0002	<0.0001	0.2643	0.1885	0.0172	<0.0001	0.0408
Flood*spp*time	0.0035	0.0221	0.0998	0.2496	<0.0001	0.0107	0.9998	0.3078	0.0717	0.9010

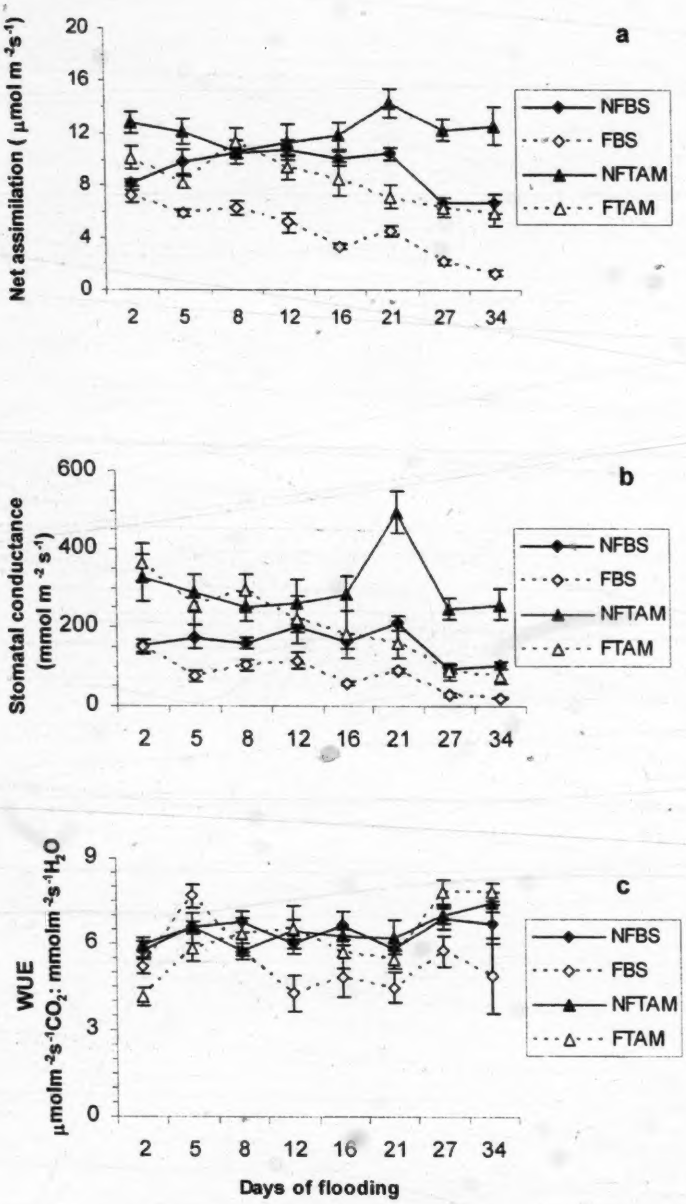

Figure 4.1. Effects of flooding on net assimilation (a), stomatal conductance (b), and water use efficiency (c) in black spruce and tamarack seedlings. NFBS = non-flooded black spruce, FBS = flooded black spruce, NFTAM = non-flooded tamarack and FTAM = flooded tamarack. Vertical bars indicate s.e. (n=5).

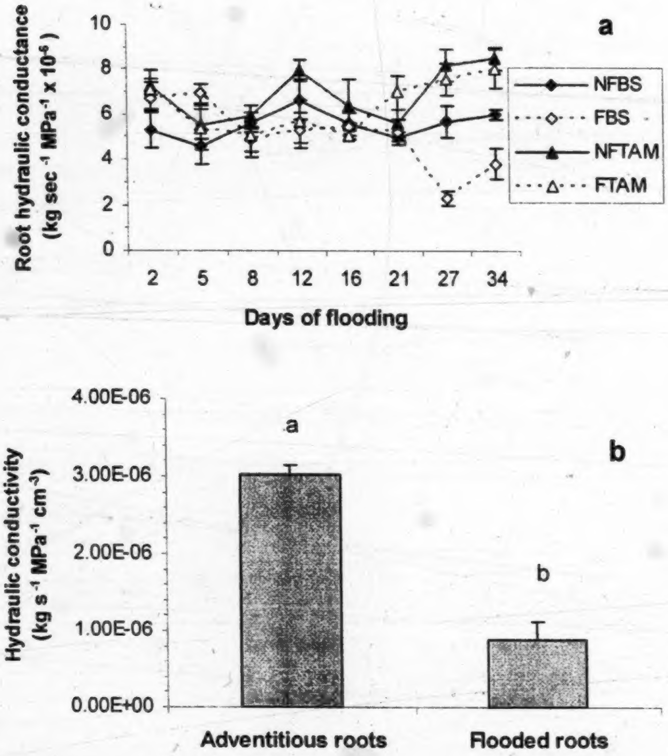

Figure 4.2. Effects of flooding on root hydraulic conductance (K_r) in black spruce and tamarack seedlings (n=5) (a), and root hydraulic conductivity (L_p) of adventitious roots (in flooded tamarack seedlings) and similarly sized flooded tamarack roots (b). Vertical bars indicate s.e. (n=4). Bars with the same letter are not significantly different at $P <$ 0.05.

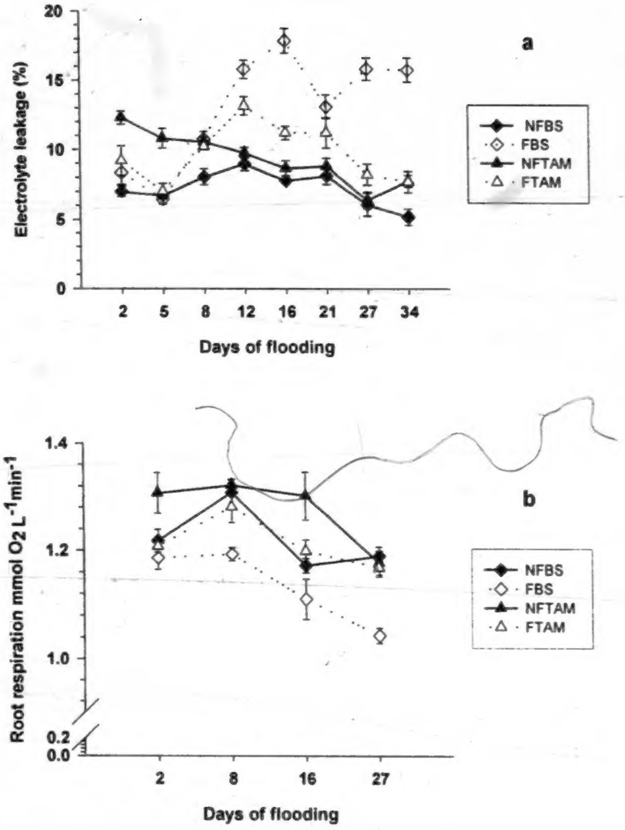

Figure 4.3. Effects of flooding on electrolyte leakage (a) and root respiration (b) in black spruce and tamarack seedlings. Vertical bars indicate s.e. (n=5).

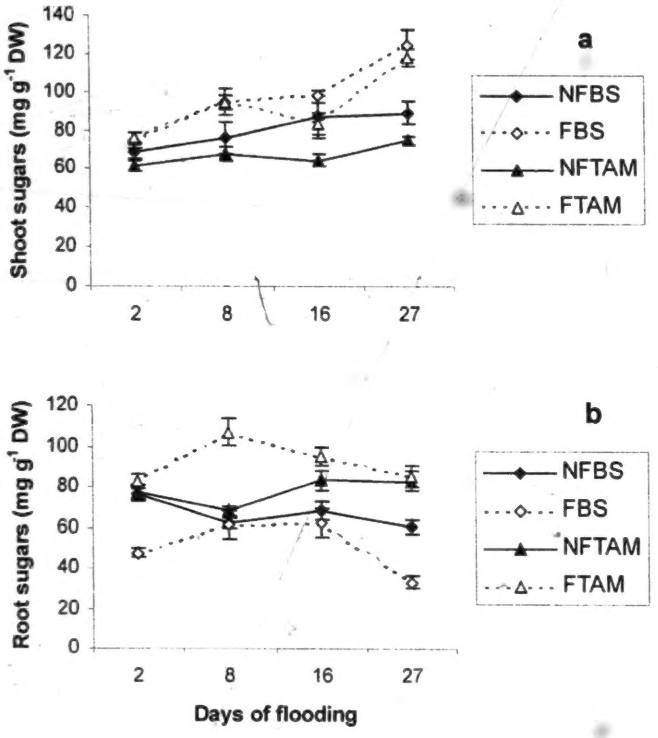

Figure 4.4. Effects of flooding on shoot (a) and root (b) sugar concentrations in black spruce and tamarack seedlings. Vertical bars indicate s.e. (n=5).

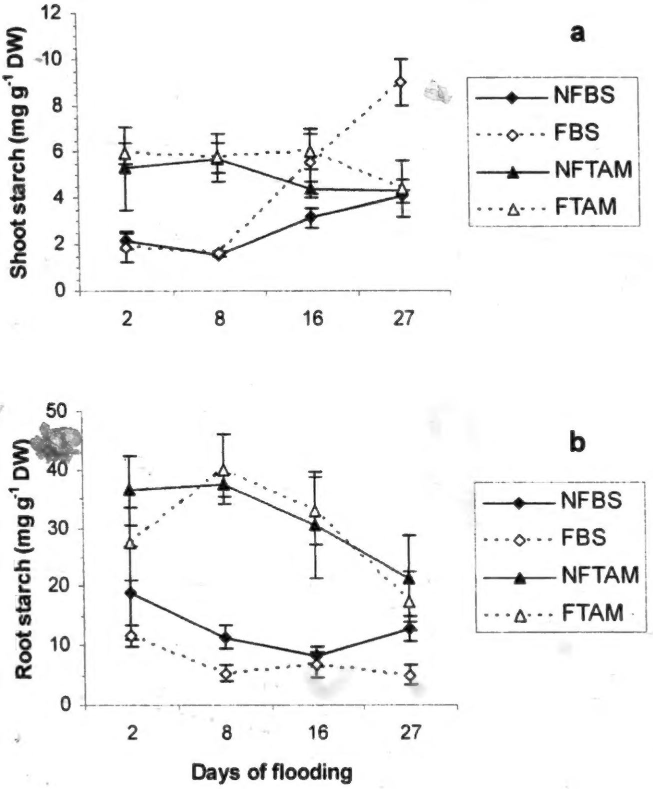

Figure 4.5. Effects of flooding on shoot (a) and root (b) starch concentration in black spruce and tamarack seedlings. Vertical bars indicate s.e. (n=5).

CHAPTER FIVE

Gas exchange and uptake of ^{15}N –labeled ammonium and nitrate in flooded black spruce and tamarack seedlings

5.1 Introduction

Nitrogen is a limiting factor for trees growing in boreal peatlands (Wollum and Davey 1975) because of the slow decomposition rate of organic matter, poor nutrient uptake due to low soil temperature (Van Cleve and Alexander 1981) and anaerobic soil conditions (Campbell 1980). Trees growing in the boreal peatlands of western Canada undergo significant fluctuation in depth of water table during the growing season (Dang et al. 1991). Oxygen availability for tree roots decreases when water tables rises close to the peat surface (Kozlowski 1984; Mannerkoski 1985). Soil flooding restricts the soil-atmosphere gas exchange resulting in depletion of soil oxygen content. Furthermore, the limited oxygen available is taken up quickly by plant roots, microorganisms, and soil reductants (Ponnamperuma 1972). Anaerobic soil conditions induce lowering of soil redox potential (Eh), which translates into a progressively greater demand for oxygen within the soil and creates an additional stress on the plant roots. With decreasing redox potential (usually at 225mV) during anaerobiosis, soil nitrate availability decreases to zero while ammonium availability increases (Armstrong et al. 1994).

Plants take up inorganic nitrogen in the form of ammonium and nitrate. Nitrate assimilation has a greater energy requirement than ammonium assimilation (Pate 1983). Once nitrate ions enter a plant cell, they are reduced to ammonium ions. The reduction process requires energy (Raven et al. 1992). A substantial amount of organic acid is required to counter OH^- generated in nitrate assimilation, resulting in a substantial amount of reduced carbon in the form of organic acid ion (Raven and Smith 1987); this process requires as much as 15% of the energy production of the plant (Chapin et al. 1987). On the other hand, ammonium assimilation, in which root respiration provides the energy (Chapin et al. 1987) and reductant required for glutamine and glutamate synthesis (Oaks and Hirel 1985), is less costly than nitrate assimilation. Tamarack has the ability to transport oxygen to its roots under anaerobic conditions and is thus able to sustain limited root respiration (Conlin and Lieffers 1993) while black spruce lacks that ability since black spruce roots are solely dependent on fermentative glycolysis in low temperature anoxic conditions. Studies have shown a positive correlation between gas exchange and foliar nitrogen of tamarack with ammonium availability and that ammonium availability is higher in wetter, lower and colder microtopographic positions in the boreal peatlands (Astridge 1996). In black spruce on the other hand, net assimilation and foliar nitrogen were correlated more with nitrate availability than with ammonium (Astridge 1996). If tamarack can sustain root respiration under anaerobic conditions, like those found in peatlands, we expect that it would be better able to assimilate ammonium than black spruce. This could explain its ability to thrive in peatlands as a deciduous conifer.

The mechanisms of NH_4^+ and NO_3^- uptake of conifers have been investigated recently in numerous studies (Kronzucker et al. 1995 a,b; 1996; Bassirirad et al. 1997; Malagoli et al. 2000; Hangs et al. 2003) and several species demonstrate a clear preference for ammonium uptake over nitrate. However, there are no studies that have examined ammonium and nitrate uptake in black spruce and tamarack during hypoxic or aerobic growing conditions.

The objective of this study was to examine ammonium and nitrate uptake rates in two boreal conifers: the evergreen black spruce and deciduous tamarack under flooded and non-flooded conditions. We used labeled $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$ since this approach allowed us to trace and quantify the amount of nitrogen that entered in to the plant under study (Nômmik 1990). We hypothesized that tamarack, compared with black spruce, can better take up ammonium during hypoxic soil conditions.

5.2 Material and methods

5.2.1. Plant material and growing conditions

One-year-old black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) seedlings were used in this experiment. Seedlings were obtained from Bonnyville Forest Nursery (6-15A) in a dormant condition and were placed at 4^0C to acclimate for a week. Afterwards, seedlings were transplanted in 1 gallon pots containing a planting medium of Pro-Mix BX and sand (1:3; Premier Horticulture Inc., Riviere-du-Loup, Que. Canada) and placed in a growth chamber with $21^oC/18^oC$ day/night temperature, 65% relative humidity and 16-h photoperiod with photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} provided by fluorescent lamps. Seedlings were watered to full saturation every other day. After about three weeks, all the buds had flushed and all the seedlings were in an actively growing stage when treatment application commenced.

5.2.2. Treatment application and experimental design

Black spruce and tamarack seedlings were supplied with 150 mg of ^{15}N per pot [simulating operational silvicultural prescription of 100 – 200 kg N ha^{-1} under field conditions (Salifu and Timmer 2003; Mugasha 1991)] as either labelled $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ (5% enriched, Sigma-Aldrich, Canada) dissolved in water. Chelated micronutrients (EDTA 42% and DTPA 13%) were applied at the rate of 0.03 g L^{-1} to prevent any micronutrient deficiency (Salifu and Timmer 2003). Half of the seedlings from both black spruce and tamarack were randomly placed in plastic tubs where flooding was imposed by submerging the seedlings to root-collar level. Perforated pots were used for the other half to provide adequate drainage for the control (non-flooded) seedlings. Non-flooded seedlings were carefully watered to full saturation level to ensure that ^{15}N fertilizer was not lost with excess watering. A total of 168 seedlings (N) were used in this experiment. The experiment was a 2 x 2 x 2 x 3 factorial design, testing form of N supply (NH_4^+ and NO_3^-), species (black spruce and tamarack), flooding (flooding and non-flooding), treatment duration (1 week, 2 week, and 3 week), and their interactions. There were seven replicates for each treatment*species combination. Gas exchange data were collected on day 7, 14, and 21 after treatment imposition. On each measurement day, net assimilation, stomatal conductance, and water use efficiency were measured on seven randomly selected seedlings from each species x treatment combination.

The statistical model was as follows:

$$Y_{ijkl} = \mu + N_i + S_j + NS_{ij} + F_k + NF_{ik} + SF_{jk} + T_l + TN_{il} + TS_{jl} + TF_{kl} + NSFT_{ijkl} + \varepsilon_{ijklm} \quad \text{......} \quad (1)$$

Where, Y_{ijkl} = Physiological parameter (e.g., net assimilation, stomatal conductance etc.), μ = overall mean, N = Form of nitrogen applied (i = 1,2). S = species (j = 1,2); F = flooding treatment (k = 1,2), T = time (l = 1,2,3); ε = error term (m = 1,2,…168). Data were analyzed using analysis of variance (using SAS version 8.1; SAS Institute Inc., Cary, NC. 1996) to determine the main and interactive effects of flooding, time and species. Means were compared for significant differences at $\alpha = 0.05$ using Tukey's test.

5.2.3. Measurements

^{15}N analysis:

At the end of each week after completing gas exchange measurements, seedlings were harvested and partitioned into shoots and roots. Roots were washed free of planting medium and both roots and shoots were oven dried for 48 h at 68^{0}C. Shoot and root samples were ground with a Wiley mill to pass a 20 mesh sieve. Then the ground samples were pulverized in a vibrating-ball mill (Restch, Type MM2, Brinkmann Instruments Co., Toronto, Ontario, Canada) as required for sample preparation for mass spectrometer analysis. Ethanol was used to clean the mill between samples after vacuum cleaning (Binkley et al. 1985). All plant samples were then run for total N and ^{15}N analysis in an elemental analyzer (NA 1500, Carla Erba Elemental Analyzer, Milan, Italy), which was connected to a continuous flow Stable Isotope Ratio Mass Spectrometer (VG 10; Middlewich, Cheshire, U.K). The mass spectrometer was comprised of an automatic Dumas system (Carlo Erba) for total nitrogen and a flow-through system for the nitrogen gas generated for isotope ratio analysis using a triple collector system.

The N isotopic ratio of samples was calculated using the delta (δ) notation as:

$$\delta\ {}^{15}N = \left[\frac{\left(atom\%\ {}^{15}N\right)_x}{\left(atom\%\ {}^{15}N\right)_{std}}\right] - 1 * 1000 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (2)$$

where, (atom%^{15}N)x and (atom%^{15}N)std are the respective N isotope ratios of the sample and the standard (0.3666, International Atomic Energy Agency, 1983) (Hauck et al. 1994). Total ^{15}N concentration (mg gdw^{-1}) in plant tissues was calculated following the equation of Salifu and Timmer (2003):

$$^{15}N\,\text{recovery}\,\% = TN\left[\frac{A-B}{C-B}\right] \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (3)$$

where, *TN* is total plant N content (mg), estimated as concentration multiplied by plant dry mass; *A* is the atom% ^{15}N in fertilized plant tissues, estimated from equation (2); *B* is atom% ^{15}N in unfertilized plant tisses (control), estimated from equation (2); C is atom% ^{15}N in fertilizer.

Gas exchange

Net assimilation (A_{net}), stomatal conductance (g_s) and water use efficiency (WUE; net carbon assimilation rate divided by transpiration rate) of seven flooded and non-flooded black spruce and tamarack seedlings were measured in each week using an infrared gas analyzer equipped with a automatic conifer cuvette (LCA-3, Analytical Development, Hoddesdon, U.K.). An artificial light source was used to supplement PAR to 1050 μmol m^{-2} s^{-1}. Net assimilation and stomatal conductance rate were calculated as described by von Caemmerer and Farquhar (1981) and expressed on a leaf area basis. The uppermost shoots of a randomly selected seedling from each species and treatment combination were placed in the cuvette for gas exchange measurements. Gas exchange and all other physiological measurements were taken on days 7, 14, and 21 after flooding imposition. Needles were carefully detached from the stem and their surface area measured by computer scanning (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA). Net assimilation and g_s were calculated as described by Caemmerer and Farquhar (1981) and were expressed on a per unit needle area basis. Gas exchange measurements were taken before mid-day to avoid the midafternoon decline in the photosynthesis rates.

5.3 Results

5.3.1. Physiological responses

In general tamarack performed better than black spruce under both flooded and non-flooded conditions having higher net assimilation (A_{net}), stomatal conductance (g_s), and water use efficiency (WUE) regardless of the form of nitrogen fertilizer. Flooding significantly reduced A_{net}, g_s, and WUE in both black spruce and tamarack seedlings fertilized with both NH_4^+ and NO_3^- (Fig. 5.1; Table 5.2). There were no significant effects of form of fertilizer on gas exchange (Table 5.2). A_{net} in non-flooded black spruce seedlings and flooded seedlings of both species fertilized with NH_4^+ and NO_3^- remained approximately the same over three weeks, while it increased over time in non-flooded tamarack seedlings. Although flooded tamarack seedlings generally maintained higher A_{net} than flooded black spruce seedlings, it was not significant except for NO_3^--treated flooded seedlings at the second week measurement. Non-flooded tamarack maintained significantly higher A_{net} than non-flooded black spruce seedlings at the second and third weeks in both NH_4^+ and NO_3^- fertilized plants.

Flooded tamarack seedlings had significantly higher stomatal conductance than flooded black spruce seedlings in both NH_4^+ and NO_3^- fertilized plants only on the first measurement week. Non-flooded tamarack had significantly higher stomatal conductance rate than non-flooded black spruce at the third week measurement in both NH_4^+ and NO_3^- fertilized plants (Fig. 5.1). Flooded tamarack had significantly higher WUE than flooded black spruce after the first week in NH_4^+ fertilized and after the second week in NO_3^- fertilized plants (Fig. 5.1). On the other hand, non-flooded tamarack had a higher WUE than non-flooded black spruce after first and second week in NH_4^+ fertilized seedlings and over the entire period in NO_3^- fertilized seedlings (Fig. 5.1).

5.3.2. ^{15}N uptake and distribution in plant tissues

Flooding significantly ($P<0.0001$) reduced uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ in both species (Fig. 5.2; Table 5.2) and there were no significant uptake differences between these two species under flooding. $^{15}NH_4^+$ uptake into roots of non-flooded black spruce increased throughout the experiment. While $^{15}NO_3^-$ uptake increased in non-flooded tamarack and $^{15}NH_4^+$ uptake remained the same. Non-flooded black spruce had a significantly higher $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake than non-flooded tamarack seedlings. Both $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake into the shoot of non-flooded black spruce was significantly higher than non-flooded tamarack throughout the entire experiment. Overall, $^{15}NH_4^+$ uptake was considerably higher than $^{15}NO_3^-$ uptake in non-flooded black spruce seedlings

(Fig. 5.2) indicating a preferential $^{15}NH_4^+$ uptake in black spruce but uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ in tamarack were similar (Fig. 5.2).

In general, nitrogen concentration was higher in black spruce than in tamarack irrespective of flooding or N fertilizer source. In $^{15}NH_4^+$ fertilized seedlings, total nitrogen concentration (expressed as mg g^{-1} dry weight) in roots of flooded black spruce was significantly higher than in flooded tamarack throughout the experiment and at the first and second week in $^{15}NO_3^-$ fertilized seedlings (Table 5.1). Total nitrogen was also significantly higher in non-flooded black spruce roots than in tamarack in both $^{15}NH_4^+$ and $^{15}NO_3^-$ fertilized seedlings (Table 5.1). Non-flooded black spruce had higher shoot nitrogen concentration than non-flooded tamarack throughout the entire experiment in $^{15}NH_4^+$ fertilized seedlings but only in the first and third week measurements in $^{15}NO_3^-$ fertilized seedlings (Table 5.1). There were no significant differences in shoot nitrogen concentration of the flooded seedlings of these two species.

Percentage recovery of the applied $^{15}NH_4^+$ in the roots of non-flooded black spruce was significantly higher than in non-flooded tamarack while there were no significant differences between the species in percent recovery of both $^{15}NH_4^+$ and $^{15}NO_3^-$ in flooded roots (Fig. 5.3). Recovery of fertilized ^{15}N in whole plant tissues ranged from 0.22% to 1.73% in flooded seedlings and from 19.48% to 64.36% in non-flooded seedlings.

5.4 Discussion

A general trend of lower net assimilation and stomatal conductance in flooded seedlings of black spruce and tamarack was observed here, and is consistent with our earlier studies

(Islam et al. 2003; Islam and Macdonald 2003). During flooding, low soil redox conditions could result in reductions of net photosynthesis due to decreased leaf water potential and reduced rubisco (ribulose-bisphosphate carboxylase/oxygenase)activity (Pezeshki 1994). A decline in photosynthesis could also be due to disruption of photosynthate transport (Wample and Thornton 1984), or leaf chlorophyll degeneration (Pazeshki 2001). Flooding induced ethylene has been implicated in the decline of photosynthesis due to the loss of photosynthetic capacity of the mesophyll (Taylor and Gunderson 1988). Flooded tamarack seedlings maintained relatively higher net assimilation than flooded black spruce seedlings throughout the experiment in both $^{15}NH_4^+$ and $^{15}NO_3^-$ fertilized plants, presumably due to their tolerance to ethylene (Islam et al. 2003). In contrast to the results of Astridge (1996), there was no effect of form of fertilizer applied on gas exchange of either species.

Although stomatal conductance of flooded seedlings rebounded somewhat during the third week of the experiment, WUE declined in both species. The decline in WUE in flooded plants suggests a higher transpiration rate relative to the net photosynthesis rate, because the stomata remained open even as photosynthesis stayed the same or increased. This suggests non-stomatal limitations to photosynthesis and poor stomatal regulation of water loss during flooding, possibly due to increased translocation of ABA and ethylene from root to leaves (Zhang and Davis 1987). The higher transpiration rate could also contribute to a gradual water stress of the plants if hypoxic conditions impaired root functioning. Flooded seedlings of both species did not exhibit any morphological adaptations (such as stem hypertrophy or production of adventitious roots) during this experiment. In another study, 18-month-old flooded tamarack seedlings produced adventitious roots after 16 days of flooding imposition (Islam and Macdonald 2003). Seedlings in this experiment experienced the same duration of flooding but they did not respond the same way. One possible explanation is that they were younger (12-months old). Failure of plants to form adventitious roots may be associated with a deficiency of appropriate enzymes or enzyme activators, the separation of reactants owing to cellular compartmentalization, a deficiency of cofactors (Haissig 1972, 1974), or an interaction between auxin and ethylene (Wample and Reid 1979).

Flooding also inhibited the uptake (expressed in mg g^{-1} dw) of both $^{15}NH_4^+$ and $^{15}NO_3^-$ in seedlings of both species. Inhibition of nutrient uptake and transport due to root dysfunction or death occurs during flooding because of highly reduced soil conditions (DeLaune et al. 1998, 1999). Often blockages in the vascular and aerenchyma systems may result from phytotoxin damages in highly reduced soils (Armstrong et al. 1996 a,b,c). Uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ was significantly higher in the root and shoot of non-flooded seedlings of both species compared to flooded seedlings. Despite this, and the higher total nitrogen concentration of its foliage, the rate of photosynthesis rate for black spruce per unit leaf area was significantly lower than tamarack irrespective of flooding treatment. The net assimilation rate of non-flooded tamarack was significantly higher than non-flooded black spruce seedlings in this study as was also reported previously (Islam et al. 2003; Islam and Macdonald 2003) under optimal growing conditions. Since tamarack has a very low leaf weight per unit area, its rate of photosynthesis per unit area or per unit N concentration is dramatically higher than in black spruce (Macdonald and Lieffers 1990). Its leaf structure, therefore, is an important contributer to its highly efficient utilization of nitrogen (Tyrrell and Boerner 1987).

However, non-flooded black spruce had significantly higher uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ than non-flooded tamarack seedlings.

This higher uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ in non-flooded black spruce compared with non-flooded tamarack could be attributed to its larger above-ground N sink than tamarack (Mugasha 1994). In his study, Mugasha (1994) attributed this larger sink to the persistent retention and higher dry mass per needle in black spruce than in tamarack. The higher uptake of ^{15}N by non-flooded black spruce and its relatively higher tissue nitrogen concentration than non-flooded tamarack did not significantly affect its physiological functioning since its net assimilation rate remained the same for the entire experiment. This could be attributed to the lower photosynthetic nitrogen use efficiency (PNUE) of the evergreens (DeLucia and Schlesinger 1995) which is associated with high specific leaf mass (SLM). The leaves of evergreens invest proportionally more N in nonphotosynthetic functions such as defensive compounds, and the leaves may also have relatively high cell wall resistance to gas diffusion (DeLucia and Schlesinger 1995). In contrast, the lower uptake of ^{15}N in tamarack likely reflects its lower demand since it has a smaller N sink and is more efficient in nutrient retranslocation (Tyrrell and Boerner 1987). As a consequence, tamarack has a lighter-weight, annually replaced canopy, in which it invests less than black spruce.

The higher uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ in non-flooded plants indicates active root and shoot growth during the experiment. A 64% recovery of the total ^{15}N applied in non-flooded black spruce and 41% in non-flooded tamarack seedlings is higher than the recovery of ^{15}N reported in other studies (Knowles and Lefebvre 1972, Salifu and Timmer 2003) suggesting a high fertilizer efficiency and effectiveness of the experimental setup. Knowles and Lefebvre (1972) reported about 8-12% recovery of applied ^{15}N (urea) in black spruce seedlings over one growing season. A higher recovery (12-19%) of applied $^{15}NH_4{}^{15}NO_3$ between 60 and 120 days after treatment application in black spruce was observed in studies of Salifu and Timmer (2003). Such differences of recovery between our study and others could be attributed to the seedlings age and stage of growth.

While non-flooded black spruce clearly demonstrated a higher uptake of $^{15}NH_4^+$ over $^{15}NO_3^-$, non-flooded tamarack seedlings had almost identical uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$, Unlike tamarack, Malagoli et al. (2000) reported preferential uptake of NH_4^+ over NO_3^- in European larch (*Larix decidua* Mill.). The higher and preferential uptake of $^{15}NH_4^+$ in non-flooded black spruce over $^{15}NO_3^-$ could be explained by the fact that the later requires more energy to assimilate. Such preferential uptake of NH_4^+ has also been observed in evergreen conifers such as white spruce (Kronzucher et al. 1995a, Kronzucher et al. 1997), loblolly pine and ponderosa pine (Bassirirad et al. 1997). In this regard, the ability of tamarack to uptake equally both NH_4^+ and NO_3^- is unique.

Although non-flooded black spruce had higher uptake of both NH_4^+ and NO_3^- than non-flooded tamarack, it failed to translate this to an increased photosynthetic performance. On the other hand, we expected tamarack to have superior ability to take up ammonium when flooded since it can transport oxygen to its roots and maintain higher root respiration than black spruce. Instead, we observed that tamarack could equally acquire both ammonium and nitrate while black spruce preferred ammonium. Since ammonium is the available form of N in wet, hypoxic peatland sites, black spruce would be at a disadvantageous position to acquire ammonium because of its inability to perform required root respiration.

In peatlands, there is a lot of variability with the season, between years and between microsites in water level, therefore, anaerobic condition will affect the form of N available. Since tamarack can take up both forms of nitrogen, it is well suited to take advantage of variations in form of nitrogen. Also its ability to sustain root respiration during flooding should give it an advantage in nutrient acquisition over black spruce.

5.5 References

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Table 5. 1. Total nitrogen concentration (mg g^{-1} dry weight) in plant tissues of flooded and non-flooded black spruce and tamarack seedlings sampled 1, 2 and 3 weeks after fertilization with ^{15}N –labeled ammonium and nitrate. Values are means (s.e.) of five seedlings. *Denotes significantly different at *P*<0.05.

Root	**Treatment**	**Species**	**1 week**	**2 week**	**3 week**
NH_4^+ fertilized	Flooded	Black spruce	10.40*± (0.52)	9.64* ± (0.27)	10.61*± (0.34)
	Flooded	Tamarack	9.14 ± (0.54)	8.08 ± (0.31)	8.07 ± (0.22)
	Non-flooded	Black spruce	12.18* ± (0.41)	12.70* ±(0.20)	14.04*± (0.39)
	Non-flooded	Tamarack	9.36 ± (0.22)	9.38 ± (0.22)	8.80± (0.36)
NO_3^- fertilized	Flooded	Black spruce	9.98* ± (0.46)	9.66* ± (0.29)	9.47 ± (0.51)
	Flooded	Tamarack	8.62 ± (0.19)	7.74 ± (0.32)	9.46 ± (0.35)
	Non-flooded	Black spruce	11.66*± (0.44)	11.90*±(0.36)	12.24*±(0.37)
	Non-flooded	Tamarack	9.10 ± (0.5[illegible]	10.34± (0.42)	9.74± (0.31)
Shoot					
NH_4^+ fertilized	Flooded	Black spruce	8.54 ± (0.24)	7.70 ± (0.14)	7.94 ± (0.14)
	Flooded	Tamarack	7.82 ± (0.14)	7.14± (0.12)	8.48 ± (0.13)
	Non-flooded	Black spruce	14.12*±(0.40)	13.38*± (0.56)	12.72*± (0.29)
	Non-flooded	Tamarack	9.60 ± (0.34)	10.36 ±(0.48)	9.90± (0.17)
NO_3^- fertilized	Flooded	Black spruce	8.48 ± (0.26)	8.18 ± (0.37)	7.78 ± (0.35)
	Flooded	Tamarack	8.98 ± (0.27)	8.84 ± (0.22)	8.60 ± (0.19)
	Non-flooded	Black spruce	12.12* ± (0.23)	11.38± (0.45)	13.28*± (0.26)
	Non-flooded	Tamarack	11.00 ± (1.11)	11.28± (0.22)	10.18± (0.18)

Table 5. 2. Results of Analysis of Variance (ANOVA) testing for main effects of form of N fertilizer (NH_4^+ and NO_3^-), species (black spruce and tamarack), flooding (flooding and non-flooding), treatment duration (1 week, 2 week, and 3 week), and their interactions on net assimilation (A_{net}), stomatal conductance (g_s), water use efficiency (WUE), root, shoot, and whole plant uptake of ^{15}N. P values are given for each response variable for the effects tested for in ANOVA.

Source	Response variables					
	A_{net}	g_s	WUE	Root ^{15}N uptake	Shoot ^{15}N uptake	Whole plant ^{15}N uptake
Fertilizer	0.0760	0.1132	0.4316	0.0001	0.0001	0.0001
Species	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Flooding (trt)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time	0.0001	0.0001	0.0001	0.0001	0.0015	0.0001
Fert*spp	0.8929	0.8274	0.8663	0.0008	0.0074	0.0004
Fert*trt	0.0908	0.9288	0.0009	0.0006	0.0002	0.0001
Fert*time	0.1585	0.0144	0.1611	0.0530	0.0253	0.4863
Spp*trt	0.0001	0.0200	0.0049	0.0001	0.0001	0.0001
Trt*time	0.0001	0.0094	0.0044	0.0001	0.0025	0.0001
Fert*spp*trt	0.5167	0.6152	0.7697	0.0003	0.0129	0.0003
Fert*spp*time	0.4049	0.3923	0.3376	0.0102	0.2359	0.0712
Fert*trt*time	0.0227	0.0042	0.1540	0.0565	0.0286	0.4977
Spp*trt*time	0.0001	0.0001	0.2332	0.0001	0.0450	0.0001
Fert*spp*trt*time	0.0510	0.0413	0.1121	0.0118	0.2601	0.0849

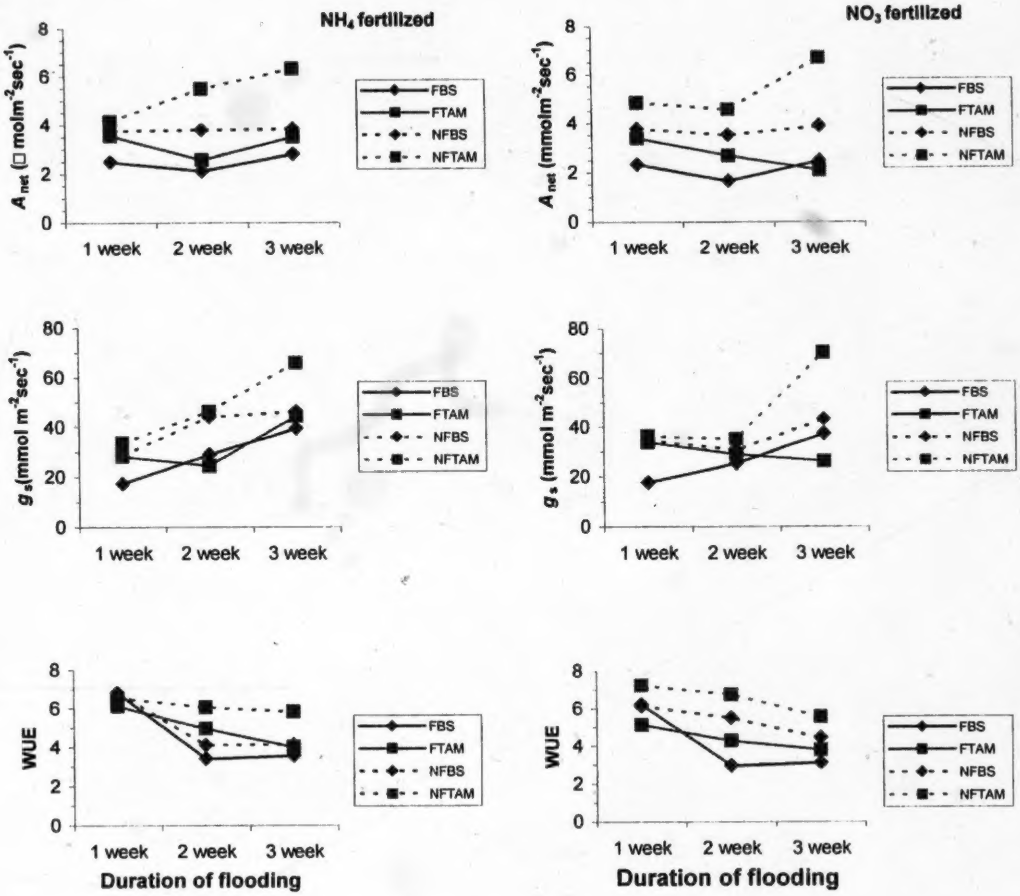

Figure 5.1. Net assimilation, stomatal conductance and water use efficiency of flooded and non-flooded black spruce and tamarack seedlings 1, 2 and 3 weeks after fertilized with ^{15}N –labeled ammonium and nitrate. NFBS = non-flooded black spruce, FBS = flooded black spruce, NFTAM = non-flooded tamarack, and FTAM = flooded tamarack. Values are means (s.e.) of seven seedlings.

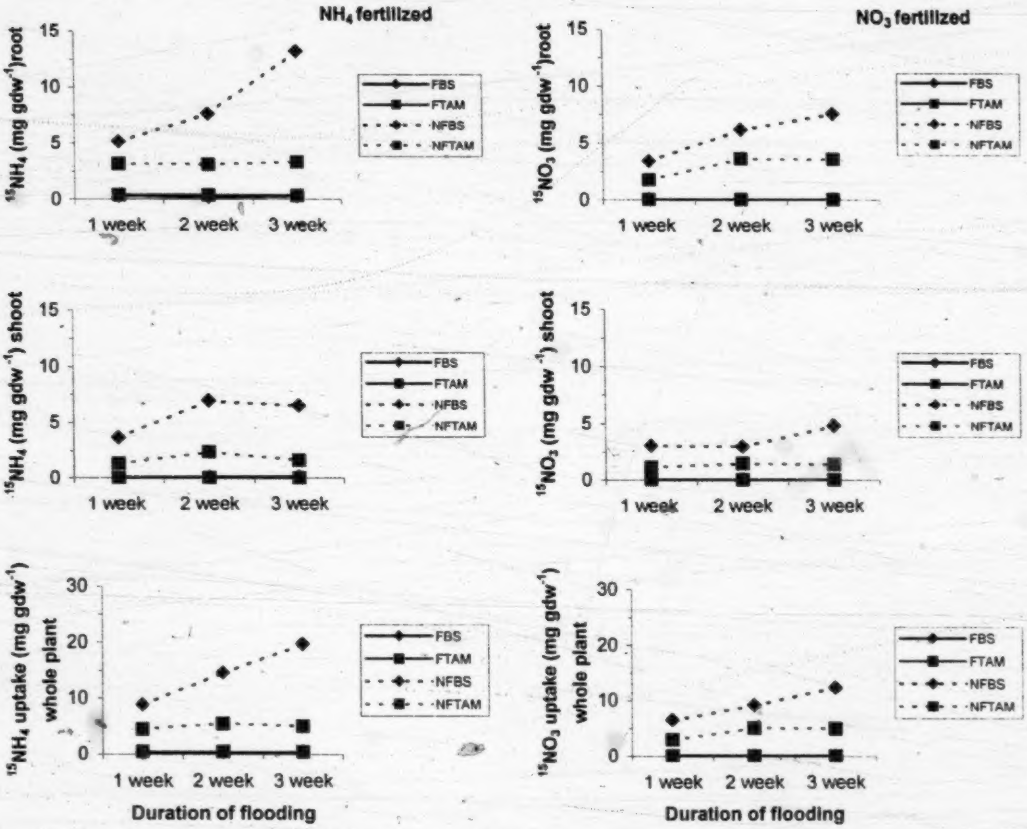

Figure 5.2. Uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ (mg g^{-1} dry weight) in plant tissues of flooded and non-flooded black spruce and tamarack seedlings sampled 1, 2 and 3 weeks after fertilized with ^{15}N –labeled ammonium and nitrate. Values are means (s.e.) of five seedlings.

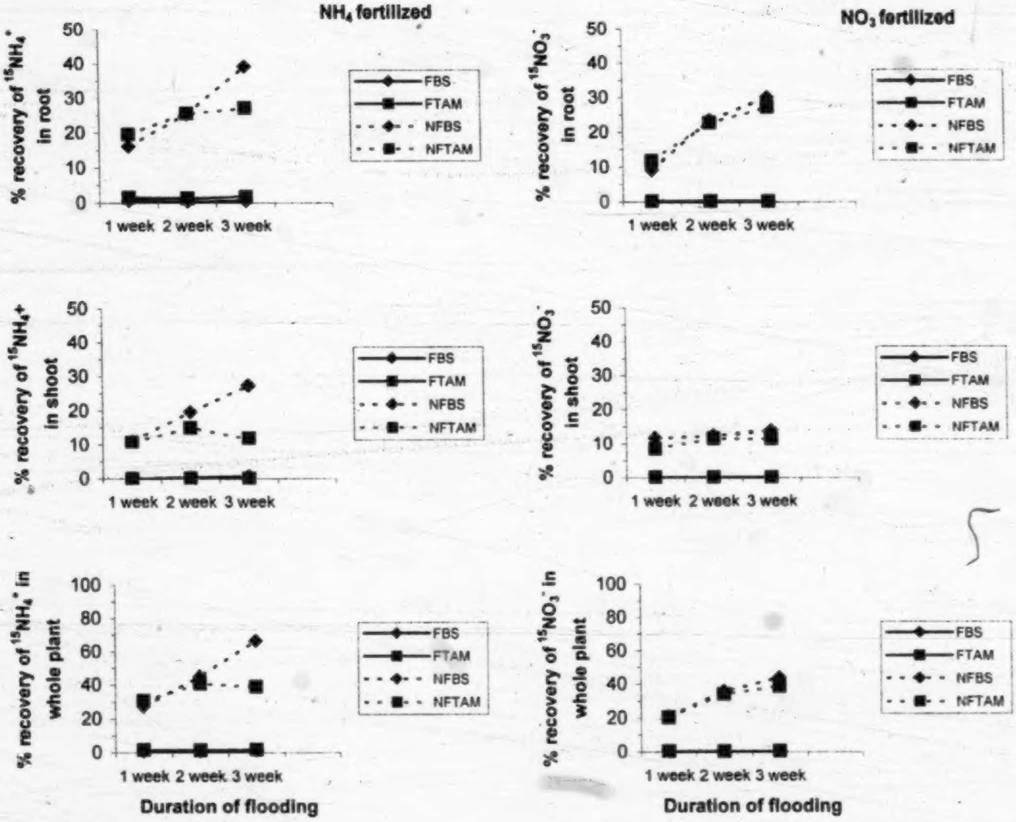

Figure 5.3. Percent (%) recovery of applied 15N (mg g-1 dry weight) in plant tissues of flooded and non-flooded black spruce and tamarack seedlings sampled 1, 2 and 3 weeks after fertilized with 15N –labeled ammonium and nitrate. Values are means (s.e.) of five seedlings.

CHAPTER SIX

Effects of variable nitrogen fertilization on growth and biomass partitioning in black spruce and tamarack seedlings

6.1 Introduction

Black spruce and tamarack are the dominant tree species in the boreal peatlands of northern Alberta (Macdonald and Lieffers 1990) and posses contrasting growth habits. Black spruce exhibits fixed growth (preformed growth) i.e., preset the number of cells and needles in prior fall and subsequent growth is limited only to those needles. In other words, the process of bud set in the previous fall determines growth in the subsequent year. Tamarack exhibits heterophyllous growth. Its short shoots have determinate growth (which displays growth similar to those in black spruce) and long shoots display indeterminate growth (i.e., presets bud in the prior fall and further apical growth occurs during the growing season and sets bud again in the fall), thus allowing a greater degree of variation of annual growth in response to conditions during the growing season (Clausen and Kozlowski 1967).

The boreal forests in the Northern Hemisphere are typically dominated by evergreen trees (Wolfe 1979). The dominance of evergreen trees in harsh forest environments has been attributed to the greater annual net carbon gain (Mooney 1972) and more efficient use of nutrients (Chapin 1980) by evergreen tress compared to species with deciduous leaves. The greater leaf longevity of evergreens reduces the annual carbon and nutrient requirement to produce new foliage (Chabot and Hicks 1982). In peatlands, low nutrient requirement and the conservative use of available nutrients by evergreens are thought to be particularly important because nutrient availability is low due to anaerobic conditions caused by high water table (Campbell 1980) and the effect of low soil temperature on decomposition, mineralization, and nutrient and water uptake (Van Cleve and Alexander 1981).

Along with most evergreen conifers, tamarack, a deciduous conifer, occurs throughout the boreal forests and often dominant on wetter peatland sites ((Jeglum and He 1996, Tilton 1977, Kenkel 1987). Tyrrell and Boerner (1987) found that relative growth rates and nutrient growth efficiencies are similar for black spruce and tamarack, but resorption of nitrogen is higher in tamarack. In contrast, tamarack exhibited greater height growth (Mead 1978) than black spruce, even on crowded sites and under very limiting conditions (Montague and Givnish 1996; Bares and Wali 1979). Tamarack has a very low leaf weight per unit area, its rate of photosynthesis per unit area or per unit N concentration is dramatically higher than in black spruce (Macdonald and Lieffers 1990) and has higher total carbon gain than black spruce (Mugasha 1992).The high nutrient-use-efficiency of tamarack and its efficient retranslocation of nutrients were also reported by Mugasha (1992).

Tamarack, being a deciduous conifer, has a lower root construction/maintenance cost when soil becomes very dry, wet or cold (Givnish 2002) since it can reduce transpiration rates significantly in adverse conditions. Furthermore, tamarack, being deciduous, requires no leaf respiration during the unfavorable season (Givnish 2002). Most larches exhibit lower fine root growth than evergreen conifers; presumably because of greater carbohydrate requirements for construction of a full complement of foliage compared to the single age-class of foliage produced by evergreen conifers (Lyr and

Hoffman 1967). Therefore, tamarack is also likely to allocate less carbon to below-ground growth. Due to less investment of nitrogen for defensive compounds in leaves, tamarack has a lower needle weight per unit area than black spruce (Section 5.4). The lighter-weight canopy of tamarack allows for greater allocation to height growth (Matyssek 1986; Tyrrell and Boerner 1987; Gower and Richards 1990; Montague and Givnish 1996).

Edaphic conditions in boreal peatlands are generally poor because of low nutrient availability, cold soils and anaerobic conditions due to high water table. These aspects, however, can be highly variable among microsites or over time as a result of microtopography and fluctuations in water table. Nitrogen (N) is the most limiting nutrient in the boreal forest (Wollum and Davey 1975). Nitrogen deficiency often inhibits plant productivity (Cetiom-Inra 1983) by reducing the number of leaves and leaf area and the reduced nitrogen content of the leaves (Osman et al. 1977). Infertile soils generally result in a lower maximum rate of photosynthesis because leaf nitrogen levels are suboptimal (Mooney and Gulmon 1979). Thus, soil infertility also reduces the absolute seasonal differences in net photosynthesis and would favor the dominance of evergreens in boreal forests because they are generally better adapted to more extreme sites (Givnish 2002). However, with increasing soil fertility the dominance shifts from evergreens to deciduous plants (Fox 1992, Aerts et al. 1991, Nams et al. 1993, Givnish 2002). We hypothesized that the growth flexibility (due to deciduous habit and heterophyllous growth) of tamarack enables it to adjust with changing edaphic conditions thus confering a greater total carbon gain (total plant dry wt.) explaining its ability to thrive in conditions where evergreen conifers are thought to have an advantage.

6.2 Materials and methods

6.2.1 Plant material and treatment application:

Black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) seeds were obtained from the Alberta Tree Improvement and Seed Center (ATISC acc #4042; see also 3.2.1). Seeds were stratified for three weeks at 4^0C and seeds were germinated in Petri-dishes. One week after germination, seedlings were transplanted to Spencer-Lemaire (300ml) containers (Spencer-Lemaire Industries Ltd. Edmonton, AB. Canada) containing (1:1:1; peat:moss:vermiculite) planting medium Pro-Mix BX (Premier Horticulture Inc., Riviere-du-Loup, Que. Canada) and placed in a controlled growth chamber with 21°C/18°C day/night temperature, 65% relative humidity and 16-h photoperiod with photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} provided by fluorescent lamps. Seedlings were watered to run off every other day. Application of treatment began when seedlings were eight weeks old.

6.2.2 Treatment application and experimental design:

Seedlings from both black spruce and tamarack were fertilized once a week with high (100 ppm) or low (10 ppm) nitrogen fertilizer supplied as NH_4NO_3 as the main source of nitrogen. The other sources of macronutrients included KCl, $MgSO_4$, KH_2PO_4, $CaCl_2$, and $FeCl_3$. The micronutrients included H_3BO_3, $ZnSO_4$, Na_2MO_4, $MnSO_4$, $CaCl_2$, and $CuSO_4$.

The fertilizer treatment combinations were as follows:

Species	Nutrient treatment	
	1st growing season	2nd growing season
For both black spruce and tamarack	High	High
	Low	High
	High	Low
	Low	Low

A 2 x 4 factorial design was used to conduct the experiment with control seedlings held under either high or low nutrient supplies for the entire period. The first set of measurements (including net photosynthesis, stomatal conductance, water use efficiency, total plant dry weight, root: shoot ratio) were taken after the seedlings grew for twenty weeks.

After destructive harvesting was completed at the end of the growing season, the remaining seedlings were subjected to winter hardening by gradually reducing the growth chamber temperature and photoperiod. Plants were placed in a cold room with 10^{0}C temperature and 10 h photoperiod for six weeks then in 4^{0}C and 8 h photoperiod for eight weeks. Seedlings from both species set buds during this time. After that, seedlings were brought back to 15^{0}C temperature and 10 h photoperiod for two weeks. During this overwintering period seedlings were not fertilized. Thereafter, seedlings were transplanted to 12.7 cm pots with Pro-Mix BX (as above) and returned to the original growing conditions (21°C/18°C day/night temperature, 65% relative humidity and 16-h photoperiod) and grown under the experimental treatment condition for the second growing season for 24 weeks.

Final measurements of the same physiological and morphological parameters were taken after the second growing season. Data were evaluated by analysis of variance using SAS version 8.1 software (1996; SAS Institute Inc., Cary, NC) to determine the main and interactive effects of fertilizer and species. Means were compared for significant differences at $P < 0.05$ by Tukey's test.

6.2.3 Measurements of net assimilation (A_{net}), stomatal conductance (g_s) & water use efficiency (WUE) and nitrogen analysis:

Net assimilation (A_{net}), Stomatal conductance (g_s) and water use efficiency (WUE; net carbon assimilation rate divided by transpiration rate) of the black spruce and tamarack seedlings (treated with either low or high nitrogen) were measured using an open-system infrared gas analyzer (IRGA) (LCA-3: Analytical Development Company Ltd. Hoddesdon, U.K.) equipped with a conifer cuvette. An artificial light source was used to supplement the PAR to 1050 μmol m^{-2} s^{-1}. The uppermost shoots of a randomly selected seedling from each species and treatment combination were placed in the cuvette for gas exchange measurements. Needles were carefully detached from the stem and their surface areas measured by digitizing the scanned images (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA). Net assimilation and stomatal conductance rate were calculated as described by von Caemmerer and Farquhar (1981) and expressed on a leaf area basis. Gas exchange measurements were taken before mid-day to avoid the midafternoon decline in the photosynthesis rates.

After completing gas exchange measurements, plants were destructively harvested and plant dry weight was obtained by placing samples in an oven at 68^0C for 48 h. Oven dried shoot samples were ground in a Willey mill to pass a 20 mesh screen. Samples were digested in concentrated sulphuric acid followed by oxidation with hydrogen peroxide (Lowther 1980). Total N digests were determined with an autoanalyzer (Technicon Instruments 1977) and expressed as a concentration (percent dry weight).

6.3 Results

Overall, after the first growing season, shoot, root, total dry weight, S:R ratio, net assimilation (A_{net}), stomatal conductance (g_s) and water use efficiency (WUE) were higher for both species under the high nitrogen treatment compared to low nitrogen. The photosynthesis rate per unit leaf area was significantly ($P<0.0001$) higher in tamarack compared to black spruce seedlings after one growing season in high-nitrogen (H) (Table 6.1; Figure 6.1). However, there were no significant differences in photosynthesis rate between these two species under low-nitrogen (L) (Table 6.1; Figure 6.1). For tamarack, under H, A_{net} was 39% higher than under L whereas the H treatment only resulted in an 10% increase in A_{net} of black spruce (spp*trt P = 0.096; Table 6.1; Fig. 6.1). Overall, there were no significant differences in stomatal conductance between black spruce and tamarack in either low or high nitrogen fertilization. There was a significant species* treatment interaction for stomatal conductance in tamarack. Stomatal conductance remained essentially the same in tamarack under H and L, whereas for black spruce, g_s was 26 % higher under L compared to H nitrogen. Overall, WUE was greater for tamarack than black spruce. In black spruce WUE was similar under both low and high nutrient conditions while it increased by 66% in tamarack under high nitrogen compared low nitrogen. Shoot dry weight, root dry weight, total plant dry weight and shoot: root ratio were also significantly higher in tamarack (Figure 6.2) irrespective of fertilizer treatment. A significant species*treatment interaction was due to the fact that tamarack showed a greater increase in shoot, root, and total plant dry weight in response to the H (vs. L) fertilizer treatment. In contrast, S:R ratio of tamarack was similar under both H and L fertilizer treatment while for black spruce it increased in response to H (vs. L) fertilizer.

After the second growing season, there were many differences between the species in the way they responded to the four treatment combinations. Likewise, after the first growing season, A_{net}, shoot, root, and total plant dry weight were higher in tamarack than in black spruce. In contrast to the first year results, S:R ratio was generally lower for tamarack than black spruce and species differences in g_s and WUE differed between treatments. There were no significant differences in photosynthesis between tamarack and black spruce in LL (low nitrogen in both seasons), LH (low nitrogen in the first season followed by high nitrogen in the second season), and HL (high nitrogen in the first season followed by high nitrogen in the second season) treated plants. However, tamarack responded positively to the HH treatment (high nitrogen in both first and second growing season) with a significantly ($P<.0032$) higher rate of photosynthesis than black spruce (Table 6.1; Figure 6.3). Stomatal conductance was significantly higher in LL treated ($P<.0073$) black spruce but lower in LH treatment ($P<.0065$) compared with tamarack (Figure 6.3). There were no differences in stomatal conductance between black spruce and tamarack in HL and HH treatment. Water use efficiency was significantly ($P<.0164$) lower in black spruce than tamarack in the LL treated plants while it was higher ($P<.0013$) in black spruce (vs. tamarack) in LH treatment. There were no differences in WUE between black spruce and tamarack in HL and HH treatment (Figure 6.3).

Both species responded similarly to the increase from L to H nitrogen in the second growing season (LH compared to LL) with a substantial increase in A_{net}, but this only translated into relatively higher total plant mass for black spruce. The increase to H nitrogen resulted in a decline in g_s for black spruce with a corresponding increase in WUE. In tamarack, on the other hand, g_s increased with the move to H nitrogen in parallel to the increase in A_{net} such that WUE was similar for LL vs. LH. With the move from high to low nitrogen (HL vs. HH), both species showed lower A_{net}, g_s and WUE although the differences were larger for tamarack. Correspondingly tamarack showed lower shoot, root, and total dry weight in HL as compared to HH while black spruce showed no significant differences. Neither species showed significant differences in S:R ratio between HL and HH treatments.

Shoot dry weight, root dry weight and total plant dry weight of both black spruce and tamarack increased progressively from the LL, LH, HL to the HH treatment (Figure 6.4). The effects were more pronounced in tamarack than black spruce. Shoot: root ratio did not change much for tamarack in all the treatments while black spruce had a higher root: shoot ratio in LH compared with other treatments (Figure 6.4).

6.4 Discussion

Gas exchange data presented here were collected at the end of first and second growing seasons and represents only a snapshot of the physiological status of plants at those times. On the other hand, the growth data, which were also collected at the end of first and second growing seasons, are a reflection of cumulative response to the treatment.

Overall, tamarack showed a high degree of flexibility in gas exchange and growth in response to varying nutrient availability. It had a remarkable positive response to high N (vs. low) in a single growing season whereas black spruce responded only slightly. While neither species responded significantly to one year of improved nitrogen availability (LH) (except for increased A_{net}), tamarack showed the ability to take advantage of two years of high nutrient availability with a dramatic increase in growth. Application of high nitrogen (vs. low) fertilization made little difference for black spruce in biomass accumulation after the first growing season but tamarack responded positively by increasing its shoot and root mass by 42 % and 32% respectively (Figure 6.2).

Due to the inherent constraints (such as fixed growth in black spruce) in the ability of plants to adjust with variable growing conditions, the responses of plants to environmental variations are critical for their growth and survival. Small scale variations in microhabitat exist within boreal peatlands (Astridge 1996). Nutrient availability and root zone conditions are variable within a given year and also between years. Availability of nutrients in the form of nitrate and ammonium is influenced by anaerobic conditions in the peatlands. However, the ability of a species to adjust and respond quickly to these changes confers a competitive advantage to that particular species since variation in the physical environment influences distribution (Beatty 1984), fitness and competitive ability (Latham 1992) of a species.

Nitrogen deficiency during the growing season can seriously inhibit plant productivity (Cetiom-Inra 1983) by limiting the number of leaves and leaf area and reducing nitrogen content of the leaves (Osman et al. 1977). Nitrogen fertilization has been reported to increase foliar nitrogen content in both tamarack and black spruce in drained minerotrophic peatland sites while significantly increasing net assimilation only in tamarack (Mugasha et al. 1993; Mugasha 1992). An increase in N availability also significantly increased photosynthetic rates in *Kandelia candel* L. (Kao et al. 2001).

Our results in the first year show that increased supply of nitrogen resulted in higher foliar nitrogen concentration (2.59% dry weight) in black spruce but did not make any difference in A_{net} compared with those that had received low nitrogen (Figure 6.1). In contrast, tamarack seedlings fertilized with high nitrogen had higher foliar nitrogen concentration (2.66 % dry weight) and A_{net} by 39% compared with low nitrogen fertilized seedlings. Similarly, application of high nitrogen for two consecutive growing seasons increased foliar nitrogen in both black spruce (2.39 % dry weight) and tamarack (2.73% dry weight) causing a significant increase in A_{net} compared to black spruce (Fig. 6.3).

Somewhat surprisingly, both species responded positively to improved N availability in the second year, in terms of A_{net}, but neither responded strongly in terms of growth. This may reflect a lag associated with building tissue N concentration and balancing S:R biomass after the first low N growing season. The most dramatic growth response to an increase in N availability in the second year was a decline in S:R in black spruce. It has been observed that plants reduce investment in roots when nutrient availability improves. This opposite response may reflect a lag caused by the low nutrient availability in the first growing season and the greater availability of resources that could be directed towards root growth as a result of higher A_{net}. Black spruce demonstrated a conservative strategy of maintaining g_s level while A_{net} increased in response to improved N availability. This suggests a release from non-stomatal limitations to photosynthesis. In tamarack, on the other hand, g_s increased along with net assimilation under improving N availability suggesting both stomatal and non-stomatal limitations on A_{net} was involved.

It was expected that tamarack would perform better than black spruce when nutrition regime improved (LL vs. LH). While it accumulated greater biomass than black spruce, it did not actually exhibit any substantial change when nutrient regime changed from low to high in the second season. However, other reports suggest both tamarack and black spruce had increased foliar nitrogen content as a result of nitrogen fertilization in drained minerotrophic peatland sites, but that net assimilation was significantly higher only in tamarack (Macdonald and Lieffers 1990). Similarly Macdonald and Yin (1999) found that tamarack exhibited a significantly improved growth in response to improved edaphic conditions.

It appears that the recent (second seaso[illegible] application of nitrogen (LH and HH) had greater effect on A_{net} (measured at the end of second growing season) than any holdover from the first season fertilizer regime (Figure 6.3). Seedlings of both species that had low nitrogen application in the second season had lower A_{net} than those that had high rate of N application second season (regardless of first season application). Nitrogen deficiency also caused a reduction in photosynthesis and stomatal conductance in *Amaranthus powellii* Wat. (Hunt et al. 1985). A decline in rubisco (ribulose-bisphosphate carboxylase/oxygenase) content during nitrogen stress has been thought to cause such reduction in photosynthesis (Jolivet and Morot-Gaudry 1984).

Water use efficiency has been reported to decline in six perennial species under low nitrogen supply (Arp et al. 1988). Persistent low nitrogen (LL) treatment also reduced WUE in both species in our study. This reduction was attributed to stomatal closure, which appeared to be related to downregulation of photosynthesis. Chapin et al. (1988) reported an increase in the abscisic acid content leading to a reduction of the stomatal opening during nitrogen deficiency. While WUE remained the same for both black spruce and tamarack under low nitrogen fertilization at the end of first growing season, it increased significantly only in tamarack under high N application after the first growing season. Among first season low N plants (LL and LH treatments), WUE for LH (measured at the end of second growing season) was significantly lower in tamarack than black spruce. The opposite was observed in LL treatment (Figure 6.3). Both tamarack and black spruce responded positively to N by increasing A_{net} with increased nitrogen fertilization in the second year (LH). Since black spruce was conservative in its response by keeping the g_s rate even, it gained an advantage in WUE over tamarack. There were no differences in WUE between these two species in first season high nitrogen applied plants (HL and HH treatments).

In response to a decline in N availability (HL vs. HH), both species showed reduced A_{net}, g_s and WUE. Although the treatment differences were greater for tamarack, it still maintained higher A_{net} than black spruce under both treatments. The decline in N availability had a large negative effect on A_{net} and growth of tamarack. This was because it showed such a dramatic positive response to two years of high N availability.

Interestingly, there were no differences for black spruce between the HL and HH treatments interms of growth although A_{net} was much higher in HH than in HL. This suggests its limited ability to respond to changing nutrient availability. Indeed black spruce growth was remarkably uniform across all four nutrient treatments.

Tamarack had the same S:R ratio in both HL and HH suggests no preferential allocation to roots when N availability declined. This also supports efficient N uptake and utilization in tamarack. The higher S:R ratio in the first year in tamarack over black spruce reflects initial preferential allocation to shoot. In the second year, black spruce had overall higher S:R ratio which contrast with existing literature. Black spruce seedlings invested more in roots under nutrient limiting conditions (L) compared to the conditions of higher nutrient supply while root: shoot ratio remained the same for tamarack in both low and high fertilizer regime (Fig. 6.2). Shoot:root ratio increased in black spruce when nitrogen application was low in both seasons (LL) compared to LH. The increase in root:shoot ratio during low application of nitrogen was due to plant's increased investment in roots to acquire more nutrients. There were no differences for either species in shoot: root ratio in both HH and HL treatments. However, there were differences between species, with black spruce having more roots than tamarack. The probable reason that the two first season high treatments (HL and HH) did not differ, was because there was a reservoir of nitrogen both in the plant and in the soil sufficient to meet plant needs.

In comparing seedlings that were treated to one high and one low N year, the order of treatment (LH vs. HL) had an important influence on the responses. In terms of gas exchange, a high N treatment in the second year resulted in much higher A_{net} for both species than did any persistent effect of high N left over from the first year. Again black spruce had a conservative strategy holding g_s even as A_{net} increased under improved N conditions resulting in higher WUE. This suggests non-stomatal control of A_{net} was the driving mechanism. In contrast, tamarack increased g_s along with A_{net} when N availability increased. Thus WUE remained the same suggesting both stomatal and non-stomatal control.

A different response pattern was seen for growth in both species, the carry-forward effect of high N in the first year resulted in greater total biomass than when high N was applied in the second year. This reflects the long term benefit of an early growth advantage and this effect was more prominent in tamarack than in black spruce. Interestingly these two species responded in opposite ways to the LH vs. HL treatment in terms of their S:R ratio. As expected, tamarack S:R declined when N availability declined, reflecting increased allocation to the roots and increased when N availability increased suggesting a reduced allocation to the roots. Black spruce did the reverse since it lagged because of its inability to adjust with the change quickly. Both species grew better by taking advantage of high nitrogen availability in the first year and low in the second year compared to LH treatment suggesting a continual growth promotion even after nutrient availability decreased in the second year.

Boreal peatland microhabitats exhibit a wide range of variation in moisture and nutrient availability both spatially and temporally (Astridge 1996). Our hypothesis was that the flexibility in growth (due to deciduous habit and heterophyllous growth) of tamarack would enable it to adjust with changing edaphic conditions and confer a greater total carbon gain. Our results support this in some ways because it is clear that tamarack can readily take advantage of two years of good conditions but not one year of good conditions following a year of poor conditions. Meanwhile black spruce lacked a similar capacity to respond to good conditions.

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Table 6. 1. Results of Analysis of Variance (ANOVA) testing for main effects of fertilizer treatment (nitrogen; high vs. low after first growing season, four treatment combinations after second growing season), species (black spruce and tamarack) and their interactions on net assimilation (A_{net}), stomatal conductance (g_s), water use efficiency (WUE), root, shoot, whole plant dry weight and shoot: root ratio.

a) after first growing season:

Source	Response Variable						
	A_{net}	g_s	WUE	Shoot dw	Root dw	Total plant dw	Shoot: root ratio
Fert	0.0009	0.5805	0.0001	0.0001	0.0033	0.0001	0.0001
Spp	0.0001	0.4461	0.0001	0.0001	0.0001	0.0001	0.0001
Fert*spp	0.0257	0.3357	0.0013	0.0301	0.0055	0.0150	0.0032

b) after second growing season:

Source	Response Variable						
	A_{net}	g_s s	WUE	Shoot dw	Root dw	Total plant dw	Shoot: root ratio
Fert	0.0001	0.0056	0.0001	0.0001	0.0001	0.0001	0.0001
Spp	0.0300	0.7163	0.9234	0.0001	0.0001	0.0001	0.0001
Fert*spp	0.0961	0.0025	0.0008	0.0001	0.0001	0.0001	0.0001

Table 6. 2. Needle nitrogen concentration (percent dry weight) of black spruce and tamarack seedlings treated with high and low nitrogen. Values are the mean of six samples. s.e. values are presented in the parenthesis.

Species	**Treatment**	**%N**
Black spruce	HH	2.39 (0.08)
Black spruce	HL	2.36 (0.16)
Black spruce	LH	2.45 (0.07)
Black spruce	LL	2.08 (0.17)
Tamarack	HH	2.73 (0.09)
Tamarack	HL	1.99 (0.12)
Tamarack	LH	2.21 (0.09)
Tamarack	LL	1.84 (0.09)
Black spruce	H	2.59 (0.11)
Black spruce	L	2.44 (0.22)
Tamarack	H	2.66 (0.11)
Tamarack	L	2.53 (0.18)

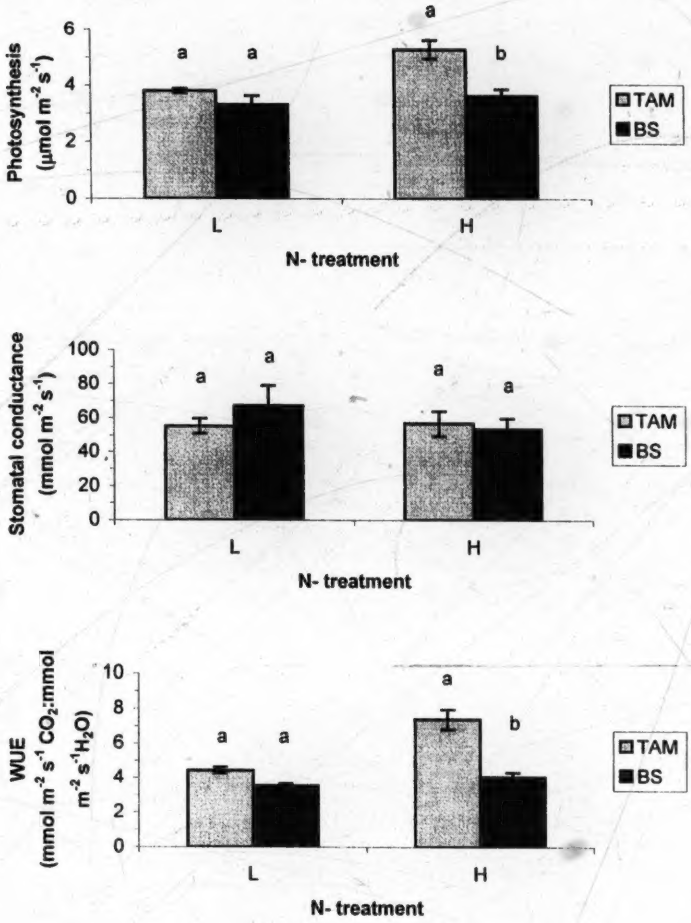

Figure 6.1. Effects of high and low nitrogen fertilizer on net assimilation, stomatal conductance and water use efficiency of black spruce and tamarack seedlings after the first growing season. TAM = tamarack and BS = black spruce. Values are means (s.e.) of six seedlings. Error bars represent s.e.

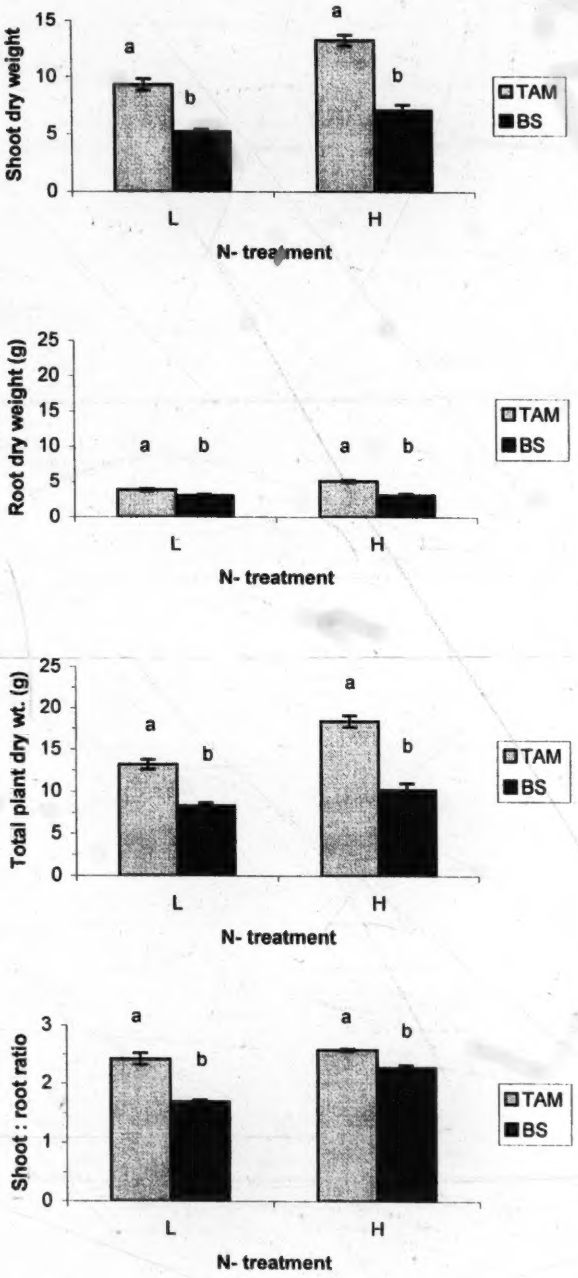

Figure 6.2. Effects of high and low nitrogen fertilizer on shoot dry weight, root dry weight, total plant dry weight and shoot: root ratio of black spruce and tamarack seedlings after the first growing season. TAM = tamarack and BS = black spruce. Values are means (s.e.) of six seedlings. Error bars represent s.e.

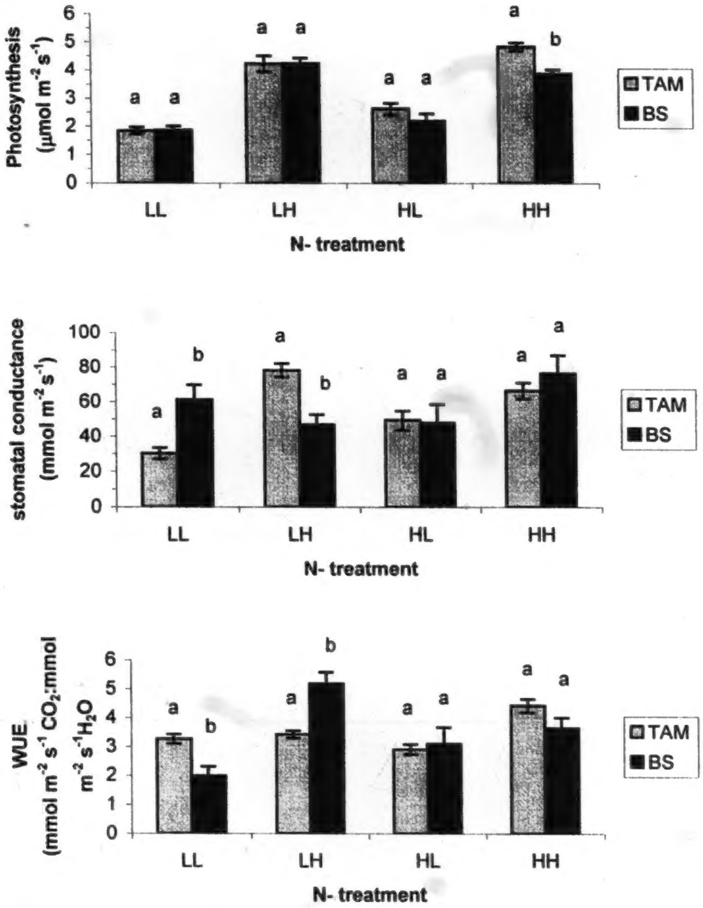

Figure 6.3. Effects of high and low nitrogen fertilizer on net assimilation, stomatal conductance and water use efficiency of black spruce and tamarack seedlings after the second growing season. TAM = tamarack and BS = black spruce. Values are means (s.e.) of six seedlings. Error bars represent s.e.

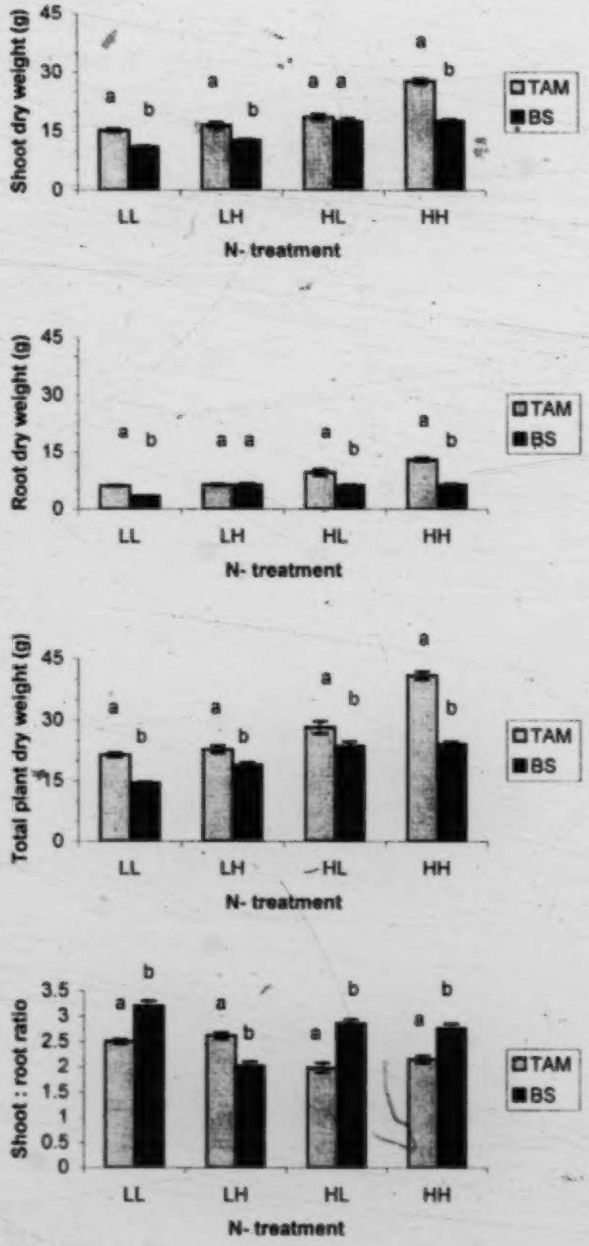

Figure 6.4. Effects of high and low nitrogen fertilizer on shoot dry weight, root dry weight, total plant dry weight and shoot: root ratio of black spruce and tamarack seedlings after the second growing season. TAM = tamarack and BS = black spruce. Values are means (s.e.) of six seedlings. Error bars represent s.e.

CHAPTER SEVEN

General Discussions and Conclusions

7.1. Flooding tolerance in black spruce and tamarack

Trees in the boreal peatlands of northern Alberta encounter a variety of stress factors, including fluctuating water table, with resultant effects on numerous plant physiological processes. Soil flooding inhibits photosynthesis and transport of photosynthates. Uptake of water and macronutrients are also reduced in flooded plants due to root mortality and/or suppression of root metabolism. Flooding alters hormonal balance in plants by triggering dramatic increases in ethylene concentration, which increases electrolyte leakage of the membranes. The general mechanisms of flood tolerance in black spruce and tamarack are presented in a schematic diagram (Figures 7.1). Flooding tolerance varies greatly among tree species and is influenced by plant age, time and duration of flooding (Kozlowski 1997). Flood tolerant plants survive soil waterlogging by complex interactions of morphological, anatomical, and physiological adaptations (Kozlowski 1997).

Black spruce and tamarack are the two dominant tree species in the boreal peatlands of northern Alberta (Macdonald and Lieffers 1990). Both black spruce and tamarack co-occur on moderately minerotrophic sites with tamarack dominating on wetter sites (Tilton 1977, Kenkel 1987, Jeglum and He 1996). Previous studies (Ahlgren and Hansen 1957) have suggested that black spruce is more flood tolerant than white spruce and white pine. However, no information was provided on how this tolerance was conferred. Thus, this current study was intended to provide a basis for understanding the different plant physiological processes that are affected by environmental stresses. It should be noted that, to date, there have been no published records available on comparative flooding tolerance mechanisms, growth dynamics or nutrient relations of these two species. Although Macdonald and Lieffers (1990) examined gas exchange performance of black spruce and tamarack on drained and undrained peatland sites, the underlying mechanism of flooding tolerance in these two species was not investigated. Therefore, the general objective of the present study was to understand the mechanisms (physiological and morphological) of flooding tolerance in black spruce and tamarack. In the first two studies, I examined the effects of flooding on gas exchange, root hydraulic conductivity, root respiration, membrane leakage, and carbohydrate allocation in black spruce and tamarack, two dominant boreal tree species.

Although the general patterns of response to soil flooding were similar in both species, there were key differences in the timing and magnitude of responses. Flooding reduced net assimilation, root hydraulic conductance, and stomatal conductance, and increased water use efficiency and needle electrolyte leakage in both black spruce and tamarack seedlings. Although flooding reduced net photosynthesis in both species, flooded tamarack maintained significantly higher net photosynthesis than flooded black spruce. A decline in net photosynthesis during flooding is attributed to a reduction in activity of the photosynthetic enzyme rubisco (Vu and Yeleonoski 1992). It is reported that stress factors can reduce activity and/or enhance rubisco degradation in crop species (Dann and Pell 1989, Lauer et al. 1989, Majumdar et al. 1991). Rubisco plays an important role in photosynthesis since it catalyzes carboxylation yielding two molecules of 3-phosphoglycerate and oxygenation that produces one molecule of 2-

phosphoglycolate (Bowes 1991). Since activity of rubisco is critical for photosynthesis, flood-induced reductions of net photosynthesis may be, at least partly, due to decreased rubisco activity. Furthermore, a reduction in stomatal conductance and net assimilation could also be due to restricting shoot water loss since root hydraulic conductance declines in flooded plants. Flood induced reductions in root hydraulic conductance have been linked to root dysfunction and/or death (Pezeshki 2001). Anderson (et al. 1984) attributed the decrease in hydraulic conductivity under oxygen-deprived conditions to an occlusion of xylem vassels by debris of the rotting root system and restricted axial water movement through roots. In our study, visible reduction in the root mass due to root mortality was evident and was partly responsible for reduced root water uptake. The mechanisms by which flooding caused reduced root water uptake is not clear, however, modifications in the root anatomy (North and Noble 1995), often observed in environmentally stressed plants, might have had an inhibitory effect on root hydraulic conductance.

Results from my first experiment (Chapter III) suggest that flooding tolerance in tamarack was due to its ability to adapt morphologically (by producing stem hypertrophy), maintenance of higher root hydraulic conductance (K_r) and withstanding the flood-induced ethylene accumulation and its resultant effects on physiological processes. Flooded black spruce seedlings had higher levels of membrane damage, a higher degree of necrotic needles and also were more sensitive to exogenous ethylene compared to flooded tamarack seedlings. In contrast, ethylene had little adverse effect on gas exchange in tamarack seedlings and increased root hydraulic conductivity. Ethylene mediated increases in root hydraulic conductance are attributed to enhanced protein phosphorylation (Raz and Fluhr 1993), which in turn enhances aquaporin activity and increases root hydraulic conductance. Aquaporins regulate water flow in plants through membranes during growth, development and stress responses (Cheng et al. 1997). Increased K_r has been shown to be a key factor underlying the enhanced growth performance and net photosynthesis of *Coffea canephora* Pierre ex Froeh. plants under conditions of excess water (Fahl et al. 2001).

Further examining the role of carbohydrate allocation pattern and root respiration provided more insight into the flooding tolerance mechanism of these two species (Chapter 4; Fig. 7.2). Over-wintered (18-months old) flooded tamarack seedlings were able to produce adventitious roots, which enhanced their root hydraulic conductance as also observed in other species (Hook and Scholtens 1978, Tsukahara and Kozlowski 1985). Production of adventitious roots has also been attributed to high nutrient-uptake efficiency of flood tolerant species (Kozlowski 1997). Tamarack has an indeterminate growth pattern and thus it is more flexible in allocating carbon to either below or-above structures. The higher allocation of carbohydrate to the roots could have facilitated production of adventitious roots since emergence of adventitious root has been shown to be positively correlated with carbohydrate content (Veierskov 1988).

Carbohydrates are the primary energy storage compounds in plants and their allocation pattern and translocation rate plays an important role in flooding tolerance (Huang and Johnson 1995). A high reserve of carbohydrate in the root has been shown to confer flooding tolerance in some plants (Barclay and Crawford 1983). High root sugar concentration in flooded tamarack might also have contributed to the maintenance of their higher root respiration. A higher respiration rate, on the other hand, in flooded tamarack, also sustained higher root hydraulic conductance since continued water uptake is oxygen dependent because both the active ion uptake needed to maintain an osmotic gradient across the root (Rowe and Beardsell 1973) as well as membrane hydraulic conductivity (Glinka and Reinhold 1962) are known to require oxygen.

7.2 Nutrient uptake and utilization

Flooding alters soil nutrient availability (Pezeshki et al. 1999). Despite morphological and anatomical adaptations, various nutrient deficiencies and toxicities may occur in flooded plants (Tanaka and Yoshida 1970). Inhibition of nutrient uptake and transport due to root dysfunction or death occurs because of highly reduced soil conditions (DeLaune et al. 1998, 1999). Often blockages in the vascular and aerenchyma systems may result from phytotoxin damages in highly reduced soils (Armstrong et al. 1996 a,b,c). Although nutrient uptake (both NH_4^+ and NO_3^-) of both black spruce and tamarack was suppressed by flooding, they differed from each other in non-flooded conditions. The limited ion uptake in flooded black spruce and tamarack was made possible by the internal oxygen supply (John et al. 1974). However, if flooding continued, partial anoxia in the root zone could reduce further solute intake (Gibbs et al. 1998).

Under non-flooded conditions, black spruce showed a clear preferential uptake for NH_4^+ over NO_3^- while tamarack was able to take up both ammonium and nitrate equally (Chapter 5). The different nutrient acquiring strategies in black spruce and tamarack is of great ecological significance. Conditions in the boreal peatlands are such that the rise of the water table during the growing season makes ammonium more available. Astridge (1996) reported a positive correlation between ammonium availability and net assimilation in tamarack, while in black spruce nitrate availability was similarly correlated with net assimilation. Although non-flooded black spruce had a significantly higher uptake of both NH_4^+ and NO_3^- than non-flooded tamarack in the present study, it failed to translate this to an increased photosynthetic performance. On the other hand, the ability of non-flooded tamarack to acquire both ammonium and nitrate equally might be competitively advantageous for them to perform well in conditions of the boreal peatlands where variation in microsite and nutrient form availability changes over time due to prevailing anaerobic conditions because of periodic flooding.

Tamarack also showed a flexible growth and physiologically responded positively to a varied availability of nutrients (Chapter 6). While both black spruce and tamarack showed decreased growth under low nitrogen supply, tamarack maintained higher biomass, photosynthesis, and water use efficiency than black spruce. High nitrogen treatment in the second year increased net assimilation in both species (regardless of first year treatment). There were no differences in the photosynthesis rate for both species in LH treatment while tamarack had a relatively higher rate of photosynthesis than black spruce in HL treatment. Application of high nitrogen for two consecutive growing seasons increased foliar nitrogen in both species but tamarack seedlings were also able to dramatically increase net photosynthesis and growth compared to black spruce.

Given the inherent constraints (such as fixed growth in black spruce) in the ability of plants to adjust with variable growing conditions and take advantage of better conditions, the responses of plants to environmental variations are critical for their growth and survival. Small-scale variations in microhabitat exist within boreal peatlands (Astridge 1996). Nutrient availability and root zone conditions are variable within a given year and also between years. Availability of nutrients in the form of nitrate and ammonium is influenced by anaerobic conditions in the peatlands. Drainage of peatlands has been reported to improve oxygenation and increase nutrient availability to plants in the peatlands. However, the ability of a species to adjust and respond quickly to these changes confers a competitive advantage to that particular species. Our results suggest that tamarack is better able to utilize available nitrogen (regardless of quantity applied) and increase net photosynthesis and biomass accumulation than black spruce.

7.3. Limitations of current research and future research approach

The possible combined effects of flooding and low temperature stress (which was not studied here) are likely to be important to understand the growth dynamics of both black spruce and tamarack and therefore, are worthy of further research. All the experiments reported here were conducted in an environmental growth chamber, which has certain limitations, as it did not actually reflect the seasonal and temporal dynamics of temperature and photoperiod present in nature. The results of this study should be considered indicative of possible responses of black spruce and tamarack under natural conditions.

Although beyond the scope of this study, future research could investigate the potential role of phytoglobins in flooding tolerance in these species. Some plants can prevent the build-up of potential phytotoxins that enhances their survival under flooded conditions (Visser et al. 2003). A specific type of haemoglobin (Phytoglobin) may play such a role by detoxifying nitric oxide formed during hypoxia of root tissues (Visser et al. 2003). Furthermore, phytoglobin may also regenerate NAD$^+$, thereby serving as an alternative to fermentation as its source (Dordas et al. 2003).

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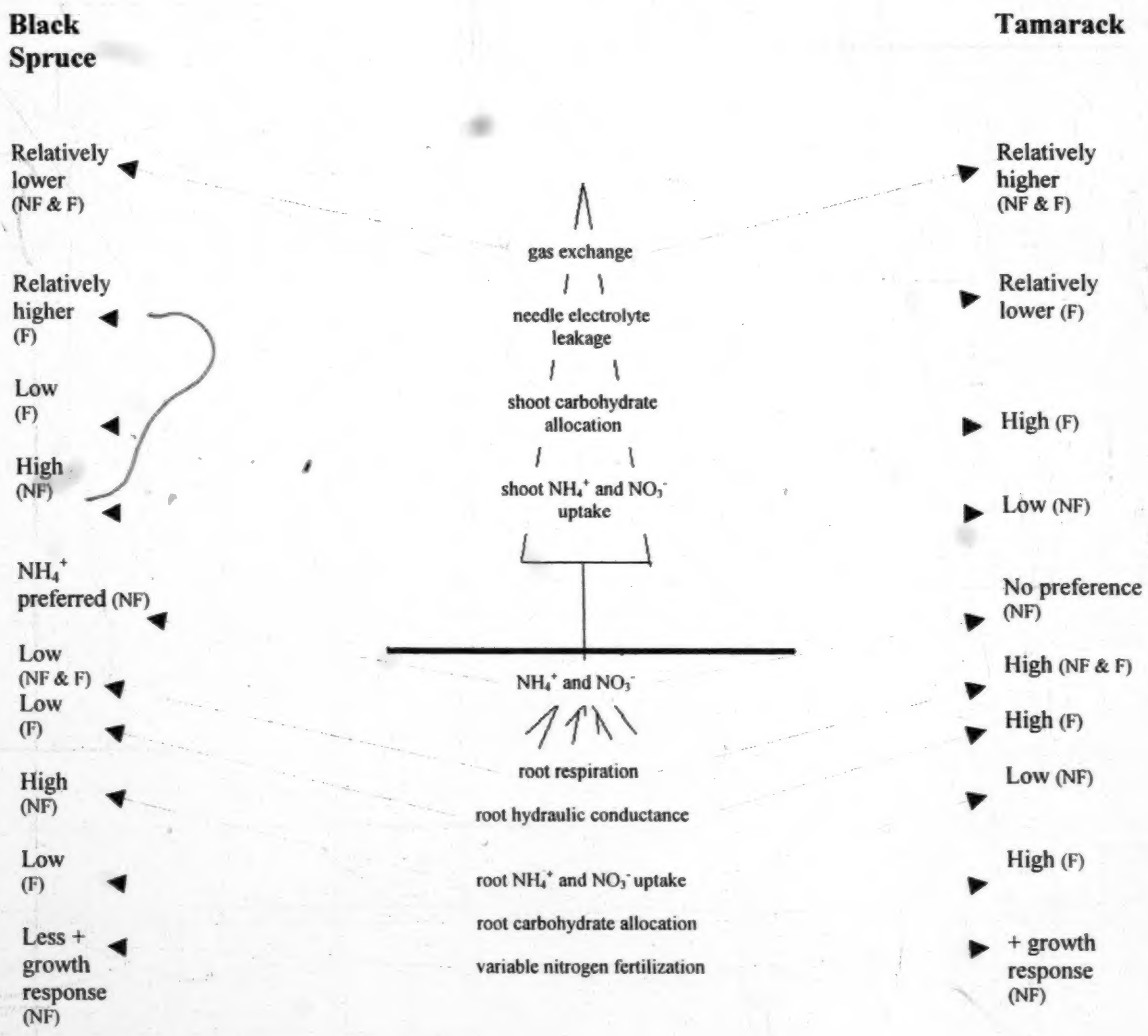
Black Spruce
Tamarack
Relatively lower (NF & F)
Relatively higher (NF & F)
gas exchange
Relatively higher (F)
Relatively lower (F)
needle electrolyte leakage
Low (F)
High (F)
shoot carbohydrate allocation
High (NF)
Low (NF)
shoot NH₄⁺ and NO₃⁻ uptake
NH₄⁺ preferred (NF)
No preference (NF)
Low (NF & F)
High (NF & F)
Low (F)
High (F)
NH₄⁺ and NO₃⁻
root respiration
High (NF)
Low (NF)
root hydraulic conductance
Low (F)
High (F)
root NH₄⁺ and NO₃⁻ uptake
root carbohydrate allocation
Less + growth response (NF)
+ growth response (NF)
variable nitrogen fertilization

* NF = non-flooded condition; F = flooded condition

Figure 7.1. Comparison of physiological responses in black spruce and tamarack in response to flooding, variable nitrogen fertilizer and form of nitrogen.